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CHARACTERISTICS AND KINETICS OF BIOLOGICAL FIXED FILM REACTORS

Billy H. Kornegay and John F. Andrews

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The kinetics of biological slime films were investigated using six completely mixed, annular reactors. Design criteria for trickling filters were formerly based on experience and guesswork, so this investigation attempted to determine the kinetics of a simple system to provide a better basis for establishing trickling filter design criteria. A simple glucose and mineral base substrate was fed to microorganisms attached to the walls of the 6 annular, completely mixed reactors. The reactors were operated first in parallel, for a basic investigation of biological film kinetics, and second, in series, to approximate plug flow. Completely mixed reactors eliminated channeling, dissolved oxygen and substrate gradients, and the interdependence of velocity and flow. Film development was uniform, and liquid channeling did not occur. This investigation revealed that biological films develop in three distinct stages: (1) logarithmic growth which lasted until the active thickness was reached; (2) linear growth, between the active thichness and the plateau thickness; and (3) zero growth, as all new cells are washed away. The active film thickness averaged 70 and was independent of D.O. Kinetic equations were developed which showed good correlation between theoretical and observed data. On this basis, a trickling filter design equation, based on the kinetics of the process, was developed. (Lowry-Texas)

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DEPARTMENT OF ENVIRONMENTAL SYSTEMS ENGINEERING
CLEMSON UNIVERSITY

CHARACTERISTICS AND KINETICS OF FIXED-FILM BIOLOGICAL REACTORS

by

Billy H. Kornegay
and
John F. Andrews

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THE CHARACTERISTICS AND KINETICS OF FIXED-FILM BIOLOGICAL REACTORS

I. INTRODUCTION

BACKGROUND

The trickling filter is a biological process which has been used for the treatment of biodegradable domestic and industrial wastes since it was introduced by the Massachusetts State Board of Health in 1889 (1 p 81, 2 p 2). In spite of the widespread application of the process since its inception the search for a rational design procedure and an adequate understanding of the process still continues.

Earlier investigators were plagued with a number of problems, but the major difficulty was the absence of mathematical models necessary to describe a host of hydraulic and biological phenomena which occur as the waste stream "trickles" over the biological film which adheres to the media within the filter. Therefore, many investigators were forced to use the "black box" approach in the formulation of design equations. That is, an effort was made to predict the removal of organic waste by the trickling filter with little or no consideration given to the biological reactions which are responsible for the degradation of the organic waste. While this approach was adequate for the past, it appears that more efficient and rational design procedures are required to meet the growing pollutional loads of the future. Since efficiency of design is contingent upon process knowledge, future design procedures should have a rational and fundamental basis.

A basis for a rational approach to the design of biological treatment processes was introduced by Monod (3) when he presented a theory for the continuous culture of microorganisms. While this theory has been successfully employed for modeling slurry reactor systems (4,5,6), it has not been applied to fixed-film reactors such as acetic acid generators or trickling filters. Since trickling filters, like other biological processes, depend upon the action of microorganisms for the removal of waste organics, it should be possible to describe this removal in terms of biological growth.

OBJECTIVES

The broad objective of this investigation was to describe the kinetics of attached microbial films as applied to the trickling filter process. Within this broad framework the specific objectives were to determine:

1. The effect of substrate concentration on the rate of substrate removal, and organism growth.
2. The effect of oxygen concentration on the rate of substrate removal, organism growth and thickness of the active layer of microorganisms.
3. The formulation and experimental verification of a mathematical model for the removal of substrate in a single stage, completely mixed, fixed-film reactor.
4. The formulation of a mathematical model for the removal of substrate in a plug-flow, fixed-film reactor.
5. The experimental approximation of the plug-flow model by operating completely-mixed reactors in series.

In addition, preliminary studies were made to determine the effect of mixing speed on the residence time distribution of the liquid, the thickness of the biological film and the rate of substrate removal.

PLAN OF STUDY

A theoretical investigation of this nature often necessitates the use of a simplified substrate and reactor system rather than one which is similar to the actual process. For this investigation a simple substrate, consisting of glucose and a mineral base, was fed to completely-mixed, annular reactors in which a mixed population of microorganisms was attached to the walls. A basic investigation of biological film kinetics was conducted by operating the reactors in parallel, and the plug-flow condition associated with trickling filters was then approximated by operating six reactors in series.

Completely-mixed, annular reactors were selected for several reasons. Preliminary studies showed that an even distribution of liquid and biological film was difficult to maintain in an inclined plate reactor. In addition, a suitable method could not be found for accurately determining the thickness of the biological film. The process was further complicated because of dissolved oxygen and substrate gradients which existed along the reactor length. The same objections were applicable to the packed-tower reactor plus the additional problem presented by the interdependence of velocity and flow. These problems were eliminated by the use of the completely-mixed, annular reactor. Film development was uniform and since the reactors were completely filled, liquid channeling did not occur. Sampling was simple and each variable could be controlled or changed without affecting other parameters. Of course, this simplified system and substrate negates the use of the numerical data for design, but if the kinetics of biological films can be described, even for a simple system, then a greater understanding of biological films will have been gained and a possible foundation established for a rational design of trickling filters.

II. LITERATURE REVIEW

GENERAL

As a prelude to this investigation a literature search was conducted to obtain information dealing with or relating to the trickling filter process. While the majority of the material reviewed originated in sanitary engineering publications, such related fields as biology, microbiology, chemical and bioengineering were also consulted. A special effort was made to obtain information dealing with the continuous culture of microorganisms, but was not included in this review. Since continuous culture theory has not been applied to filter design it was felt that a reiteration of the various symposia (7,8,9) and available reviews(10-16), at this point would be redundant. Therefore, only literature dealing with the trickling filter or related topics is presented in this section. This material has been divided into the following major areas for ease of presentation:

1. History and early development
2. Mathematical formulations
3. Film characteristics and activity
4. Oxygen requirements and ventilation
5. Environmental and operating conditions

HISTORY AND EARLY DEVELOPMENT

As civilization has progressed the development of major population centers has made waste disposal more essential and complex.

However, until the latter half of the 19th century the major objective of waste disposal was the prevention of a nuisance condition rather than disease (1). Therefore, the methods usually employed were dilution or irrigation. This attitude began to change in 1855 when Dr. John Snow, in the now famous case of the Broad Street Well (17, p. 157), showed that there was a definite relation between polluted waters and disease. As specific disease producing organisms were isolated (*Salmonella typhoas*, Eberth, 1880; *Vibro comma*, Koch, 1883; *Endamoeba histolytica*, Losch, 1875; *Shigella dysenteriae*, Shiga, 1896) (17, p. 172) the need for removing both putrescible organics and pathogenic organisms became apparent.

It had long been noted that periods of aeration were required for irrigation fields if offensive conditions were to be avoided. This led to the belief that the applied waste was rendered inoffensive through a chemical reaction with the oxygen which was stored in the voids during the period of rest. In relation to this subject, the German chemist Justus von Liebig (18) wrote:

> In this manner, every porous body-rocks, stones, and clods of the fields, etc.—imbibe air, and therefore oxygen; the smallest solid molecule is thus surrounded by its own atmosphere of condensed oxygen, and if in their vicinity other bodies exist, which have an affinity for oxygen, a combination is effected.

Fortunately, Schlossing (19) of France expelled this belief by showing that baked sand or marbles possessed no purifying capacity, even in the presence of oxygen. Furthermore, it was found that chloroform arrested the purification powers of sand, so Schlossing concluded that the presence of organic life is required for the degradation of waste products.

A major advancement was made in England in 1870 when the work of Dr. Frankland (20) and the recommendations of the Rivers Pollution Commission were combined to formulate the intermittent downward filtration process. The following year a full scale intermittent filtration system was installed at Merthyr Tudfil, Wales, and in 1881 the success of this process was related by Denton (21). The process used by Frankland was essentially an intermittent sand filter. When experiments on waste treatment were begun at the Lawrence Experiment Station by the Massachusetts State Board of Health (22) in 1888, a similar process was employed. During the course of their research evidence was gathered which indicated that purification was not related to mechanical straining, so in 1889 a media of large washed gravel-stone was employed and the first trickling filter was born (1, p. 81, 2, 22). Although the first trickling filter was established in America the practice of applying sewage by a spray and the introduction of "false bottoms" or underdrain systems originated in England (2, p. 2). Furthermore, Chase (23) attributes the development of the trickling filter from a practical viewpoint to Corbett of Salford, England.

The second experimental trickling filter installation in America was at Madison, Wisconsin, in 1901 and in 1908 operational filters were installed at Columbus, Ohio, and Reading, Pennsylvania (2, p. 2). The design parameters used at that time were similar in concept, though different in magnitude, to the parameters used for irrigation fields, namely per capita or hydraulic loading rates. According to Venable (24, p. 131), the loading rate in 1908 was 1-1.5 million gallons per acre per day or one acre per 10,000-20,000 persons. During the 1930's experiments on high rate trickling filters by Jenks (25) and Halvorson

(26) increased the allowable loading to more than 10 MGAD. In 1936 the first high rate filter was constructed (27, p. 15). During the period from 1908 to 1940 the number of trickling filter installations in the U.S.A. increased from 2 to 1,486 (28), a clear indication of the success of the trickling filter process.

MATHEMATICAL FORMULATIONS

In spite of the early success of the trickling filter it was realized (29, p. 133) that better and more rational design procedures were required in order to relate the numerous variables to performance. The first successful attempt to derive a mathematical expression relating performance to design and operational variables resulted from an extensive study of sewage treatment plants at military posts during World War II. For this investigation the National Research Council (NRC) (30) selected certain representative treatment plants for complete study, including 34 of the approximate 300 filter installations then in service at military posts. It was found that all biological treatment processes exhibited similar characteristics, and in this regard the National Research Council reported that:

> In general it may be stated that the degree of treatment attained in each [treatment process] will depend upon the following factors: (1) magnitude of organic load treated per unit time; (2) amount of biologically active growth, whether sessile or free-floating; (3) adequacy of air-liquid interface; (4) time of contact between organic load and biological growth; (5) degree of agitation and turbulence at the interface of growth and sewage and (6) provision made for the settling of agglomerated material and detached excessive growths.

Furthermore, the National Research Council reasoned that the amount of biological growth in a filter is fixed by the dimensions of the filter and that the amount of contact between the biological growth and the organic matter depends upon the filter dimensions and the number of passes by the organic waste. This led to the conclusion that the efficiency of a filter should be a function of the organic load, W; the filter volume, V; and the number of passes by the organic waste, F. When trickling filter efficiency was plotted against the value, W/VF, the line of best fit was

$$E_1 = \frac{1}{1 + .0085\sqrt{W/VF}} \tag{1}$$

where E_1 - the fractional efficiency of a single or first-stage filter

W = the wt of 5-day BOD in the raw settled sewage (lbs)

V = the volume of filter media (acre-feet)

F = a recirculation factor.

It was pointed out that the number of passes by the organic matter, F, is not equal to the number of passes made by the recycling liquid, F'. The number of passes made by the liquid is

$$F' = 1 + R/I \tag{2}$$

where F' = the number of passes made by the liquid

R = the quantity of recycled flow

I = the quantity of settled sewage flow

while the number of passes made by the organic waste matter is

$$F = \frac{1 + R/I}{[1 + (1-p)\ R/I]^2} \qquad (3)$$

where p = a weighting factor to account for the decreasing treatability of the waste and varies from 0.81 to 0.95.

Equation (1) is generally known as the NRC formula for trickling filters although the committee noted that it also described the performance of contact aeration and activated sludge plants studied. The efficiency for second-stage filters was given by the equation

$$E_2 = \frac{1}{1 + \frac{.0085}{1 - E_1} \sqrt{W'/VF}} \qquad (4)$$

where E_2 = the fractional efficiency of the second-stage filter

W' = the BOD in the effluent of the first-stage filtration system (lbs)

Velz (31) proposed a theory of biological filtration in his "law" which states:

> The rate of extraction of organic matter per interval of depth of a biological bed is proportional to the remaining concentration of organic matter, measured in terms of removability.

This was also expressed mathematically as

$$-\frac{dL}{dZ} = KL \qquad (5)$$

which Velz noted is similar to Phelps biological oxidation "law." The integrated form was given as

$$\frac{L}{L_o} = e^{-KZ} \qquad (6)$$

where L_o = the total removable fraction of BOD

L - the removable fraction of BOD remaining at any length

Z - the depth (L)

K - a rate constant (L^{-1}).

Velz postulated that not all of the applied BOD is removable and that the removable portion depends upon the relative amount of BOD in the suspended or colloidal form as compared to the portion in true solution. In addition, it was noted that the value of L decreased with an increasing hydraulic loading rate but remained constant for organic loadings up to some limiting value. This limiting value was estimated at 1 lb. of BOD per square foot per day at 30°C. For organic loading rates greater than the limiting loadings, a linear removal was assumed.

Stack (32) treated the trickling filter as a self-regenerating absorption tower and made several assumptions similar to those made by Velz The assumptions are:

(1) Each unit depth of filter removes a constant fraction of the organic waste remaining at that depth.

(2) Not all of the BOD applied to a filter is removable.

(3) The quantity of BOD that can be removed by a unit volume has a maximum limit.

Stack termed the fraction of removable BOD absorbed per unit depth the coefficient of biosorption f., and noted that it is related to the Velz equation by the expression

$$f = 1 - e^{-K} \qquad (7)$$

Stack (32) showed that when recirculation is not practiced the organic removal may be expressed as

$$R = fL\,[1 + (1-f) + (1-f)^2 + (1-f)^3 \ldots + (1-f)^{Z-1}] \qquad (8)$$

where R = quantity of BOD removed in the filter (M)

L = quantity of removable BOD applied (M)

f = coefficient of biosorption

Z = the filter depth (L).

It should be noted that as Z approaches infinity the value of the bracketed series approaches 1/f and R approaches L.

When the applied organic loading exceeds some maximum value, which Stack defined as the saturation loading S, the removal through each unit depth is

$$R = fS \qquad (9)$$

until the quantity of organic matter remaining, L_a is less than S. The removal through the saturated portion of the filter is given by

$$R = XfS \qquad (10)$$

where X = the number of saturated unit depths.

The depth of the unsaturated portion of the filter is then equal to (Z-X) and the total removal of the partially saturated filter is

$$R = XfS + f\,(L-XfS)\,[1 + (1-f) + (1-f)^2 + (1-f)^3 + \ldots (1-f)^{Z-X-1}] \qquad (11)$$

Where recirculation is incorporated and a portion of the filter is saturated, the removal is given by

$$R = XfS + f\,(L-XfS)\left(\frac{N+1}{1 + NC_{(Z-X)}f}\right)(C_{(Z-X)}) \qquad (12)$$

where N = recirculation ratio

$C = 1 + (1-f) + (1-f)^2 + (1-f)^3 \ldots + (1-f)^{Z-1}$.

Howland (33) suggested that the time of contact, rather than depth is the important variable in filter design and expressed the Velz equation in terms of contact time to obtain

$$\frac{L}{L_o} = e^{-Kt} \qquad (13)$$

where t = time of contact (T).

In addition, he found that the time of contact is inversely proportional to the hydraulic rate of flow per unit area raised to the 2/3rd power and a direct function of depth. Sinkoff, Porges and

McDermott (34) noted that the time of contact proposed by Howland could be expressed as

$$t = 1.3\ Z\ \frac{(3v)}{g}^{1/3}\ \frac{(A_v)}{Q}^{2/3} \qquad (14)$$

where v = kinematic viscosity (L^2/T)

g = acceleration due to gravity (L/T^2)

A_v = specific surface area of the media (L^2/L^3)

Q = volumetric rate of flow per unit area (L/T).

It was found that in general the residence time of a filter could be expressed as

$$t = C\ \frac{(A_v)}{Q^n}\ nZ \qquad (15)$$

where n and C are constants and depend on the filter media and hydraulic characteristics of the system. The value of n for glass and porcelain spheres was reported to be 0.83 and 0.53, respectively.

Schulze (35) related the fraction of BOD remaining to the flow Q by the equation

$$\frac{L}{L_o} = aQ^n \qquad (16)$$

where a and n are constants. The value of "n" for a screen filter was found to be 0.67. Using this value Schulze showed that Equation 13, as proposed by Howland, could be expressed as

$$L/L_o = e^{-KZ/Q^{0.67}} \tag{17}$$

where

$$t = Z/Q^{0.67} \tag{18}$$

Schulze also stated that the efficiency of a filter remains constant with organic loading up to some limiting loading.

Eckenfelder (36) postulated that the removal in a trickling filter should be proportional to the active filter slime and the time of contact between the waste and the biological slime. It was assumed that removal follows first order kinetics and could be described by the expression

$$L/L_o = e^{-K't} \tag{19}$$

where K' = a coefficient incorporating the surface area of the film per unit volume, (T^{-1}).

In addition it was noted that the previously derived expressions relating time of contact to depth and flow could be expressed by the general equation

$$t = C\,\frac{Z}{Q^n} \tag{20}$$

where

$$C \alpha \frac{1}{Z^m} \tag{21}$$

Eckenfelder combined Equations (19), (20), and (21) to obtain the equation

$$L/L_o = e^{-[K'Z^{(1-m)}]/Q^n} \tag{22}$$

The value of "m" was reported to be zero when biological films are uniform with depth in which case Equation (22) is reduced to the more common form

$$L/L_o = e^{(-K'Z)/Q^n} \tag{23}$$

Eckenfelder also pointed out that the removal often varies from first order kinetics and decreases with concentration of time. From an analysis of published data the following retardant equation was proposed

$$L/L_o = \frac{100}{1 + \frac{CZ^{(1-m)}}{Q^n}} \tag{24}$$

This retardant action or decreasing value of K has also been observed by Howland, et al. (37).

With the exception of the NRC equation, most mathematical models presented to this point are similar in format to the basic model of Velz. However, in recent years considerable effort has been directed toward filter design, both here and abroad. Work at the Water Pollution Research Laboratory in England (38) led to the equation

$$R_Z = R_u(1-2^{-Z/Z_u}) \tag{25}$$

where R_Z = percent removal at depth Z (M/L^3)

R_u = ultimate removal in an infinitely deep filter (M/L^3)

Z = depth (L)

Z_u = depth at which $R_Z = 1/2\ R_u$(L).

In spite of the difference in format, Equation (25) provides essentially the same solution as the Velz model.

Another deviation from the first order reaction model is the equation developed by Galler and Gotaas (39) by multiple regression analysis of 322 observations. Their equation relates the BOD loading, flow, temperature, recycle, depth and effluent in the expression

$$L_e = \frac{0.31\ L_o^{1.19}\left(\frac{Q}{I}\right)^{0.28}}{(1+Z)^{0.67}\ T^{0.15}\ Q^{0.06}} \tag{26}$$

where L_e = BOD load of effluent (lbs per acre per day)

L_o = BOD load of influent (lbs per acre per day)

T = temperature (°Centigrade)

I = influent flow rate (MGAD)

Q = hydraulic flow rate through filter (MGAD)

Z = depth of filter (ft).

From Equation (26) it is predicted that the hydraulic flow, Q has little effect on filter performance. This is at variance with the findings of others (31,32,33,35,36).

Ames, Behn and Collins (40) developed a theoretical model based on an analogy to packed columns commonly used in the chemical industry. When adsorption and a first order rate of reaction were considered the organics fraction L at any filter depth, Z may be related by the expression

$$L = \beta + (L_o - \beta)\exp \frac{-R}{(R+1)} \frac{kA_v}{\rho Q} Z \qquad (27)$$

where $R = K/\alpha k A_v$

K = first order reaction constant (T^{-1})

k = mass transfer coefficient ($M/T\text{-}L^2$)

A_v = specific surface area of media (L^2/L^3)

α = equilibrium constant

β = nontransmissible fraction of BOD

ρ = liquid density (M/L^3)

Amado (41) presented an equivalent expression for the exponent in Equation (27) as

$$\frac{-R}{1+R} \frac{kA_v}{\rho} = \frac{\frac{1}{\rho}}{\frac{\alpha}{K} + \frac{1}{kA_v}} \qquad (28)$$

The additive form of the individual resistances was pointed out in Equation (28), where the overall reaction coefficient K_m may be expressed as

$$\frac{1}{K_m} = \frac{1}{RA_v} + \frac{\alpha}{K} \qquad (29)$$

where $1/kA_v$ = resistance to adsorption (S), and α/K = resistance to reaction (T).

When the overall transfer coefficient was assumed to be proportional to depth raised to a negative power and to the volumetric flow rate raised to a positive power, the equation of Ames, et al. (40), was reduced to

$$L = \beta + (L_o - \beta)\,\exp - K_1 \frac{Z^{m'}}{Q^{n'}} \qquad (30)$$

where K_1, n', and m' are constants. Through experimental studies Amado determined the constants and simplified the model to

$$\frac{L}{L_o} = 0.1 + 0.9\,\exp \frac{-0.61Z^{0.628}}{Q^{0.440}} \qquad (31)$$

Atkinson, Busch and Dawkins (42) also approached the problem from a theoretical point of view, assuming first order kinetics. It was found that the substrate removal across a differential section of an inclined plate reactor having a width W and differential length dZ, could be expressed by the equation

$$\frac{d\,(QL)}{dZ} = KLhW \qquad (32)$$

where Q = the hydraulic flow rate (L^3/T)

h = the liquid film thickness (L)

W = the reactor width (L)

K = reaction constant (T^{-1}).

The integrated form of the equation was given as

$$L = L_o e^{-k \frac{h_w Z}{Q}} \tag{33}$$

FILM CHARACTERISTICS AND ACTIVITY

Film Composition

Most investigators agree that the mass of active organisms is an important factor in determining the purification capacity of trickling filters. As a result, numerous investigations have been made in order to determine the type and quantity of organisms present in the biological slimes of trickling filters. Holtje (43) found that the types of organisms in a filter were represented by the Bacteria, Fungi, Algae, Protozoa, Nematode, Rotatoria, Chaetopoda, Crustacea, Arachnida, and Insecta. He described the biological slime as a complex society in which each group of organisms is interdependent upon the others. The branched mycelia of the fungi were credited with playing the major role in holding the slime together, and spring sloughing was attributed to the decay of this fungal mat at the expense of the accelerated growth of the bacteria.

It is also agreed that the major agents of purification in the biological filter slimes are the bacteria. However, divergent opinions exist on the importance of other microbial forms. Butterfield and Wattie (44) found that biological films contain a minimum of 300 million bacteria per cubic centimeter and that they are the same zoogleal bacteria found in the activated sludge system. It was shown that a mixture of nine pure cultures of zoogleal bacteria produced a degree of purification equivalent to a filter containing all of the flora and fauna of normal sewage. This is at variance with work by Reid and Assenzo (45) where the addition of predators to pure bacterial cultures resulted in an increase in film activity. Investigators in England (46,47) also indicate that the predatory population influences the activity of filter films, but the major benefit of this grazing fauna was attributed to a reduction of the excess mass of biological film. James (48) reported that over 90 percent of the bacterial flora of trickling filters belong to the genera Achromobacter, Alcaligenes, Flavobacterium, Pseudomonas, and Zoogloea. It was noted that the organisms in these filter growths consist primarily of Gram-negative, rod shaped bacteria similar to those found in natural waters.

Film Distribution and Accumulation

In addition to studying the type of organisms present, considerable effort has also been devoted to a study of the quantity and distribution of the filter biota. James (48) and investigators at the Water Pollution Research Laboratory (49,50) in England found that in general the biological film thickness decreases from top to bottom of the filter. It appears that the film thickness is proportional to the organic waste removal in each level, but uniform film thicknesses with respect to depth have also been reported (47). It has also been shown that much thinner thicknesses occur at the surface of the filter than at the one or two foot depth (48, 51). Heukelekian (51) investigated this phenomenon by varying the flow and found a positive relation between flow rate and film thickness. He concluded that a flushing action was not responsible for the lower film accumulation observed on the surface. However, Reid and Yang (52) found that the slime growth in a pipe decreased as the velocity was increased. In these same studies it was determined that the amount of slime growth in a pipe depends on temperature, strength of sewage, velocity of sewage flow, time and pipe roughness. Standbridge (53) also indicated that film accumulation could be controlled by the flushing action of high flow rates, and it is generally accepted that low rate filters slough intermittently while high rate filters discharge the excessive growth on a continuous basis. The effect of velocity was also evident in studies by Heukelekian (51) which showed that biofilters (flow = 6.3 MGAD) contained between 5.5 and 11.0 pounds of dry film per cubic yard of filter while the film weight of the lower rate standard filters (flow = 3.8 MGAD) varied from 8.1 to 12.0 pounds per cubic yard.

Several studies (47, 54, 55, 56, 57), however, indicate that the total bio-mass is not a good indication of film activity or filter efficiency. In studies conducted by the Water Pollution Research Laboratory in England (55) it was observed that the efficiency of an experimental filter increased until a film thickness of 120 microns (μ) was reached and thereafter efficiency remained constant. The results of this investigation are shown in Figure 1. The film thickness of 120 μ was calculated from weight measurements made on an inclined rotating tube after 20 minutes of draining. An even distribution of film with respect to tube length was assumed. Sanders (56) found similar results but observed a maximum removal at a minimum critical depth of 21 μ and noted that substrate removal decreased when this thickness was surpassed. It was postulated that feed-back of

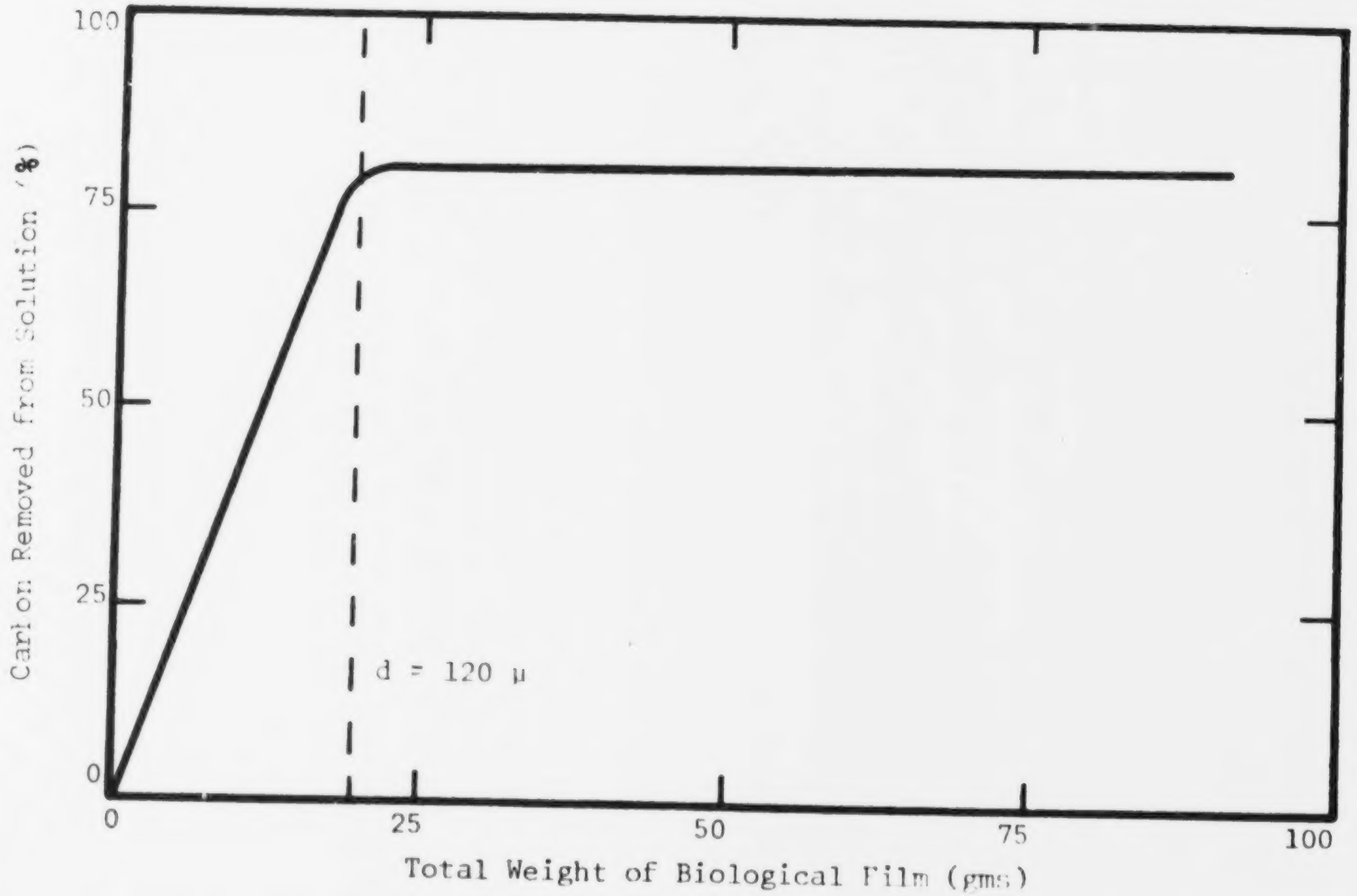

FIGURE 1. RELATION BETWEEN CARBON UTILIZATION AND TOTAL FILM ACCUMULATION

lysis or organism decay products from the anaerobic zone were responsible for this reduction in removal. Similar results have also been observed for actual filters. Hawkes (58) found that the more efficient of two similar filters was the one with the thinner film, and Jenkins and Hawkes (47) noted that the increased efficiency achieved through recirculation and altering double filtration may be due to a reduction in the accumulated film. In other experiments (57) a maximum filter efficiency was reached with a film accumulation corresponding to only 3 percent of the filter voids, although film accumulation continued until approximately 26 percent of the voids were occupied. It therefore appears that efficient filter operations do not require appreciable film accumulations.

Film Activity

The exact mechanisms involved in the purification of sewage are not well understood but obviously involve the biological films. Tomlinson (59) suggested that the waste transformation is too rapid to be explained merely on the basis of biological oxidation. However, there is evidence which shows that trickling filter films accomplish more than just coagulation or agglomeration of the organic waste. In discussing the results of an investigation of the biochemical characteristics of filter films, Heukelekian (60) concluded that considerable oxidation occurs and that filters are not just colloiders which convert non-settable solids into a settable form. In addition, it was found that proteins alone constitute 45-50 percent of the solids and it was pointed out that this must be due to protoplasmic growth made possible by the utilization of nitrogen from the sewage. In more recent studies Reid and Assenzo (45) used radiophosphorous uptake methods to study reaction rates and found that biological slimes first absorb and then assimilate the waste products removed. The rapidity with which organics are assimilated was demonstrated in a study (55) in which approximately 20 percent of the feed carbon was converted to film and 51 percent to carbon dioxide with a retention time of only 10 minutes. The remaining carbon passed through the filter and was discharged in the effluent. Ziemke (61) found that 85 to 90 percent of the BOD reduction in a test filter and final tank was due to oxidation in the filter alone.

Nitrification has also been used as an indication of film activity and filter efficiency but the process is more complex than BOD or carbon removal. Adeney (62) found that biological oxidation occurred in two stages, the carbonaceous matter being oxidized in the first and nitrification occurring in the second. Based on this report, the common belief developed that carbon is removed in the upper filter depths, followed by nitrification in the lower levels. This occurrence has in fact been generally observed in practice. However, Heulelekian (63) found that nitrification and the oxidation of carbon can occur simultaneously if nitrifying organisms and an adequate oxygen supply are available. In fact, he later determined (64) that the greatest nitrifying capacity of two filters being studied occurred in the upper two-foot level. In summarizing many of the studies on biological filtration, Stanbridge (53) suggested that the rapid oxidation of carbon in the upper filter levels may deplete the oxygen to such an extent that nitrification is inhibited. In more recent studies (65) it was found that substantial nitrification did not occur until the dissolved oxygen concentration was increased above 0.4 - 0.5 mg/l. Thereafter the increase in the rate of nitrification was proportional to dissolved oxygen until a dissolved oxygen concentration of approximately 3 mg/l was reached. Another factor credited with a decrease in nitrification was an excessive growth of biological film. In an experimental filter (66) operated at 25°C the rate of nitrification decreased by a factor of four when the film thickness increased from approximately 0.3 mm to 0.4 mm and nitrification virtually ceased when a film thickness of 0.6 mm was reached. It was surmised that the reduction in nitrification was produced by an overgrowth of the nitrifying organisms by heterotrophic bacteria. Nitrification was restored by increasing the oxygen concentration or washing off the overgrowth of heterotrophic bacteria.

OXYGEN REQUIREMENTS AND VENTILATION

Oxygen Requirements

The utilization and supply of oxygen in aerobic biological processes has received widespread attention. In studying the oxygen requirements of bacteria growing in the presence of excess substrate, Rahn and Richardson (67) found that the oxygen requirements for different genera varied. It was noted that the variation is not dependent on size alone since the species of the Enterobacteriaceae group and Pseudomonas, which all have a cell volume of approximately 0.75 cubic microns, have different oxygen requirements. At maximum growth rates Enterobacteriaceae consumed oxygen at a rate of 3 to 5×10^{-10} mg. of oxygen per cell per hour, while the genus Pseudomonas required 9×10^{-10} to 10^{-9} mg. per cell per hour. The oxygen requirements obtained for biological films based on these figures are only slightly higher than values reported by Wuhrmann (68) for activated sludge flocs, which ranged from 6 x 10^{-3} to 3 x 10^{-1} mg. O_2 per cu.cm/hr Furthermore, if a floc density of unity is assumed, the above values are also in general agreement with values reported by Muller, Boyle and Lightfoot (69). The latter investigators found the maximum oxygen demand decreased from more than 25 mg. O_2 per gram of cells per hour to less than 10 mg. O_2 per gram of cells per hour as the organism age increased from 24 to 144 hours. It was also noted that the oxygen demand is temperature dependent. Fortunately, Mueller, et al. (69) were able to clarify the apparent discrepancies previously reported for the concentration at which oxygen becomes growth limiting. It was found that a dispersed I-16-M strain of Zoogloea ramigera had a critical oxygen concentration of less than 0.1 mg/l while the critical concentration for floc particles of the same strain ranged from 0.6 to 2.5 mg/l depending upon the floc size and activity. These studies were also conducted in the presence of excess substrate. The critical dissolved oxygen concentration of most activated sludge processes is usually in agreement with the value of 0.35 to 0.40 mg/l as reported by the Water Pollution Research Laboratory in England (70).

Ventilation

Based on the removal of organic matter and the adsorption of oxygen from the gas stream, several estimates have been made of the air flow required to maintain aerobic conditions in a filter. In England (71), where filters are operated at comparatively low flow rates, it was calculated that 30 cu.ft. of air is required per cu.yd. of filter media per day under normal operating conditions. Johnson (72), using a 6 ft. depth of filter and a BOD loading of approximately 1500 lbs. of BOD per acre-ft. per day, calculated a required bulk air flow rate of 0.100 cu.ft. of air per min. per square ft. of filter. It was concluded that natural draft ventilation is normally sufficient to provide this flow rate. Piret (73) showed that the rate of air flow due to natural draft is approximately proportional to the difference between the temperature of the sewage applied and that of the outside air. It was found that a temperature differential of as little as 1°F may produce more than 30 times the required air flow. After reviewing the studies on filter ventilation it was concluded (71) that if the rate of oxidation in a filter is limited by the supply of oxygen, then the limiting rate is not the supply of gaseous oxygen to the filter but the rate at which the oxygen is transferred through the film of liquid and into the biological mass.

Absorption and Diffusion

It was experimentally determined (74) in the Water Pollution Research Laboratory that the oxygen deficit of a liquid flowing through a packed tower varied with depth according to the expression

$$-\frac{dD}{dZ} = KD \qquad (34)$$

where D = dissolved oxygen deficit (M/L^3)

Z = filter depth (L)

K = aeration coefficient (L^{-1}).

The aeration coefficient K was reported to be proportional to $1/Q^n$ where n is a constant and Q is the hydraulic flow rate. The integrated equation relating oxygen deficit to filter depth for a constant aeration coefficient was given as

$$\frac{D_1}{D_2} = e^{-K(Z_2-Z_1)} \tag{35}$$

where D_1 = dissolved oxygen deficit at Z_1 (M/L^3)

D_2 = dissolved oxygen deficit at Z_2 (M/L^3).

It should be noted that these experiments were conducted on clean gravel or glass balls coated with cotton fibers instead of a biological film. The removal of oxygen for the biological degradation of the organic waste has not been considered, nor has the decrease in the partial pressure of oxygen in the gas phase. This latter point is relatively unimportant since it has been observed (71) that the oxygen content of the gas phase in well operated filters is only slightly less than that of surrounding air. In spite of these apparent omissions, it appears that Equation (35) is a useful expression for the prediction of absorption of dissolved oxygen from the gas phase into the liquid film.

The diffusion of oxygen from the liquid phase into the biological film has received more attention in filter operations than the absorption of oxygen from the gas phase by the liquid. In 1923 Warburg (75) derived an equation which related the depth of penetration of a nutrient molecule into a living tissue with the concentration of the nutrient in the surrounding environment. Wurhmann (68) applied the work of Warburg to the biological films in trickling filters and showed that the diffusion of any nutrient, including oxygen, could be predicted by the equation

$$d = [(C_a-C_i)\ 2D_L/\alpha]^{0.5} \tag{36}$$

where D_L = diffusion coefficient (L^2/T)

d = depth of nutrient penetration (L)

C_a = concentration of the nutrient in the surrounding medium (M/L^3) (assumed to be equal to the surface concentration)

C_i = concentration of the nutrient in the innermost particle (M/L^3)

α = specific consumption rate of the diffusing molecule by the cell (M/L^3-T).

Since the critical dissolved oxygen concentration for bacterial cells may be as low as 0.1 mg/l (68, 69) the aerobic depth should vary as the square root of the dissolved oxygen concentration in the surrounding media. This, of course, is true only when the substrate is in excess so that the specific oxygen consumption rate is actually constant as assumed in the derivation of Equation (36). Tomlinson and Snaddon (76) found that the aerobic depth of film exposed to a pure oxygen atmosphere was 2.15 times the aerobic depth when exposed to air. According to Equation (36) the ratio d_{O_2}/d_{air} should have been 2.18. The diffusion coefficient varied from two-thirds to 10 times the value for the passive diffusion of oxygen into water (2×10^{-5} cm^2 - sec^{-1}). The abnormally high rate of diffusion was termed "facilitated diffusion," indicating an expenditure of energy by the microbial mass for oxygen transfer.

The Active and Aerobic Film Thickness

Tomlinson and Snaddon (76) calculated a critical thickness from accumulated film weight after 20 minutes of draining and reported a value of about 200 μ. This value is in fair agreement with the 100-200 μ thickness calculated by Wuhrmann (68) but somewhat higher than the thickness of 38-57 μ as calculated by Eckenfelder (36). Sanders (56) experimentally measured the critical depth of a biological film growing at substrate concentrations less than 20 mg/l and found the minimum critical thickness to be about 21 μ. This was taken as the minimum film depth observed when oxygen utilization became independent of film depth. The average film depth at that point was somewhat greater however and varied from 27 μ to 62 μ. It was reported that the lower depths of the film turned anaerobic. The development of an anaerobic zone in the lower film layers has also been indicated by others (35, 46, 53, 68, 69, 77). However, the presence of an anaerobic zone does not appear to offer sufficient proof to conclude that the active thickness and aerobic thickness are necessarily one and the same. Although Eckenfelder, Schulze and Sanders equate the active depth with oxygen diffusion, Wuhrmann (68) pointed out that Equation 36 also applies to the diffusion of a metabolic substrate and that in dilute media such as sewage the concentration of the substrate may be the rate limiting factor even when low oxygen concentrations exist. This is the generally accepted theory in filter design and definitely emphasized in the mathematical models previously presented. In work conducted on an experimental filter (71) it was found that the efficiency only increased about 8 percent when the composition of the aerating gas was increased from 2 to 20.6 percent oxygen. The dissolved oxygen concentrations of the liquid wastes were not given. Okun and Lynn (78) studied the oxygen utilization of biological floc over a wide range of dissolved oxygen concentrations and concluded that the rate of utilization is independent of oxygen concentration. The same conclusions may be drawn from an analysis of the data gathered by the National Research Council (30) on trickling filters. The information presently available conflicts to such an extent that the true effect of the dissolved oxygen concentration on film activity and filter performance is difficult to accurately assess.

ENVIRONMENTAL AND OPERATING CONDITIONS

Media

One environmental factor that may be considered in design is the type and size of filter media. While Reid and Yang (52) found that more porous surfaces support greater film accumulations, it has been shown (47, 54, 55, 56, 57) that thick biological films are not required for the efficient operation of filters. In comparing a plastic media with crushed rock having the same specific surface area, it was found by the Water Pollution Research Laboratory (79) that the film accumulation on the plastic media was much less than that on the crushed rock. In spite of the marked difference in film accumulation (approximately 1/3 less) the efficiency of the plastic media was only slightly less than the rock media and the difference was attributed to short circuiting. Good results have been obtained with a variety of media materials (80) and according to ASCE Manual of Engineering Practice No. 13 (81) any sound, hard, clean material that is free from dust and insoluble in the sewage constituents may be used.

A factor more important than surface characteristics is the specific surface area of the filter media. Rather extensive investigations in England (80) revealed that filter performance is a direct function of the specific surface area. Similar results have been reported by Levine, et al. (82). It has also been observed (83) that filters containing the media with the highest specific surface area also discharged the least solids. However, ponding was more prevalent with the smaller media which had high specific surface areas. Eckenfelder (36) noted that the active mass of organisms increased with an increase in the surface area, but the National Research Council pointed out (30) that the quantity of surface film is directly proportional to the area of the filter media only in the case of thin film accumulations.

Temperature

The effect of temperature on trickling filter performance has been studied by several investigators (84, 85, 86. 87) and it is generally agreed that an increase in temperature increases the efficiency of the process. The magnitude of, or the reasons for this increased efficiency are not completely understood. Temperature distributions in the flowing waste streams of filters have been attributed (84) to heat due to biological oxidations and the exchange of heat between the liquid and surrounding air. It was found that during warmer weather the sewage temperature always increased with depth, but in colder weather the temperature decreased through the first foot and the increased with depth. Because of the difference in the specific heat of water and air, the temperature of the latter has little effect on the process. Schroepfer, et al. (85) noted that a net drop in sewage temperature of more than 4 to 6°F has rarely been observed, even in the most severe weather. The relative importance of the air temperature was pointed out by a test at Excelsion, Minnesota, where it was found that a 4°F drop in the sewage temperature produced a 52°F increase in the temperature of the surrounding air as it passed through the filter.

In these studies it was found that low rate filter efficiency increased with temperature on an average of 0.62 percent per degree Fahrenheit while the efficiency of high rate filters increased only 0.34 percent per degree. However, it was noted that temperature effects should be independent of filter loading and the difference in efficiency was attributed to the method of dosing used in the filters.

Howland (86) reported that the effect of temperature upon biological oxidation is related to removal through the rate coefficient K, where

$$K_T = K_{20}C^{(T-20)} \qquad (37)$$

where K_T = rate coefficient at any temperature

K_{20}= rate coefficient at 20°C

C = a constant

T = temperature (°C).

The reported values of "C" for filters ranged from 1.025 to 1.041 with an average of 1.032. Velz (31) found that the limiting loading increased with temperature and this was related by the equation

$$K_T = K_{20}\ 1.047^{(T-20)} \qquad (38)$$

Green, Cooper, and Jenkins (87) found that as the temperature of an experimental filter was increased from 5°C to 12.5°C the efficiency increased from approximately 50 to 80 percent removal. A further increase in temperature to 20°C resulted in an efficiency of 95 percent but no significant increase in efficiency was noted as the temperature was increased to 30°C. This appears to be at variance with the findings of Sanders (56) which showed an increase in oxygen utilization of 77 percent as the temperature was increased from 20 to 30°C.

Flow Schemes and Velocity

One point that is generally agreed upon by most investigators (31, 32, 35, 36) is that filter efficiency decreases as the hydraulic flow rate increases. This is demonstrated by the mathematical models previously presented. Although efficiency decreases with increasing flow rates it has been pointed out (51, 88) that the absolute removal, in terms of pounds of BOD assimilated per unit of time, actually increases. Based on this observation, a number of flow schemes have been proposed to increase filter efficiency. These include recirculation, series or double filtration, and alternating double filtration. The National Research Council (30) found that the practice of recycling a portion of the effluent flow through the filter provided a beneficial effect, and some schemes were found to be preferable to others. The value of recirculation has been attributed to a number of factors. Fisher (89) credited the beneficial effect to innoculation of the raw sewage and increased aeration, while Heukelekian (90) stated that the clarifier also served as an oxidation basin. The latter explanation cannot provide the complete answer since it has been observed (30) that recirculation around the filter only, also provides a beneficial effect. Based on both a literature review and experimental observations, Jenkins and Hawkes (47) concluded that recirculation involved the principle of nutritional control to limit the film depth.

Excellent results have also been obtained by operating two filters in series, or double filtration. Sorrels and Zeller (88) found that two filters operated in series are more efficient than splitting the flow to two parallel filters. Schulze (35) reached the same conclusion. The National Research Council (30) reported that a two-stage filter with a total equivalent volume one-third to one-fourth of a standard filter could provide equivalent performance. A practice used in England (46, 47, 53) is the two-stage filter scheme in which the sequence of the filters is reversed periodically. This keeps the film accumulation low and thereby prevents clogging in addition to the advantages offered by the two-stage filter system. A recent innovation which capitalized on the increased assimilative capacity of high rate filters are the deep filters. These may consist of a continuous filter bed or a number of independent beds in a vertical stack such as those proposed by Ingram (91, 92). The introduction of lighter plastic media has resulted in the installation of a number of deep filters (93, 94, 95) which have given excellent results.

Since flow and velocity are interdependent in a packed tower, velocity as such has seldom been postulated as an advantage of the recirculation or series process. However, Sanders (56) found that the oxygen utilization of biological films growing on the wall of a completely mixed reactor increased 60.3 percent when the average velocity was increased from 0.1 fps to 1.0 fps.

III. THEORY

GENERAL

The trickling filter, like other biological processes, depends upon the action of microorganisms for the removal of organic waste. In spite of the differences in geometrical design and physical operation between filters and other aerobic biological treatment processes the reactions involved are essentially the same and may be represented by the general reaction equation:

$$\text{Organisms} + \text{Substrate} + \text{Oxygen} \rightarrow \begin{array}{l}\text{New Cells} \\ + \text{ Metabolic} \\ \text{Waste Products}\end{array} \qquad (39)$$

The above reaction occurs as the liquid waste stream flows over the filter media where the organic waste products are adsorbed and subsequently oxidized by the growth of microorganisms which are attached to the filter media. In view of the fact that the microbial population of a trickling filter is responsible for the ultimate removal of these wastes, it should be possible to describe this removal in terms of microbial growth by the theory of continuous culture of microorganisms.

PLUG FLOW MODEL

The trickling filter is a multiphase, packed tower and as such must be treated in a different manner than a suspended reactor system, which is normally considered to be homogeneous. This requirement stems from three major facts previously reported or observed for trickling filters. First, the organisms and substrate do not occupy a common volume as is the case for slurry reactor systems. Therefore, any relation between organisms and substrate must be made on a mass basis. Second, the entire mass of attached microorganisms is not active in the removal of soluble organics. This latter point was clearly indicated in the work of Sanders (56) and investigators at the Water Pollution Research Laboratory in England (55) and is illustrated in Figure 2. It should be noted that the active[*] film thickness d constitutes only a portion of the total film thickness h. Third, the volume of a trickling filter has no meaningful relation to the liquid volume, and can only be related to the volume of organisms through the area: volume ratio of the filter media. With this understanding of the trickling filter, a mathematical model can be derived based on known facts and the following assumptions:

1. Plug-flow is achieved in the liquid phase.
2. Substrate utilization due to sources other than the attached microbial film is small and may be neglected.
3. The apparent yield Y remains constant with depth.
4. Removal is described by a saturation function which incorporates the effect of diffusion and growth rate.

Levenspiel (96, p. 107) stated that since the composition of the fluid varies along the length of a plug-flow reactor, a material balance must be made for a differential element of volume. Consider

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[*] The term "active" is used throughout this text to denote that zone of organisms produced before substrate removal becomes independent of film thickness and is not intended to imply that a distinction can be made between viable and nonviable organisms.

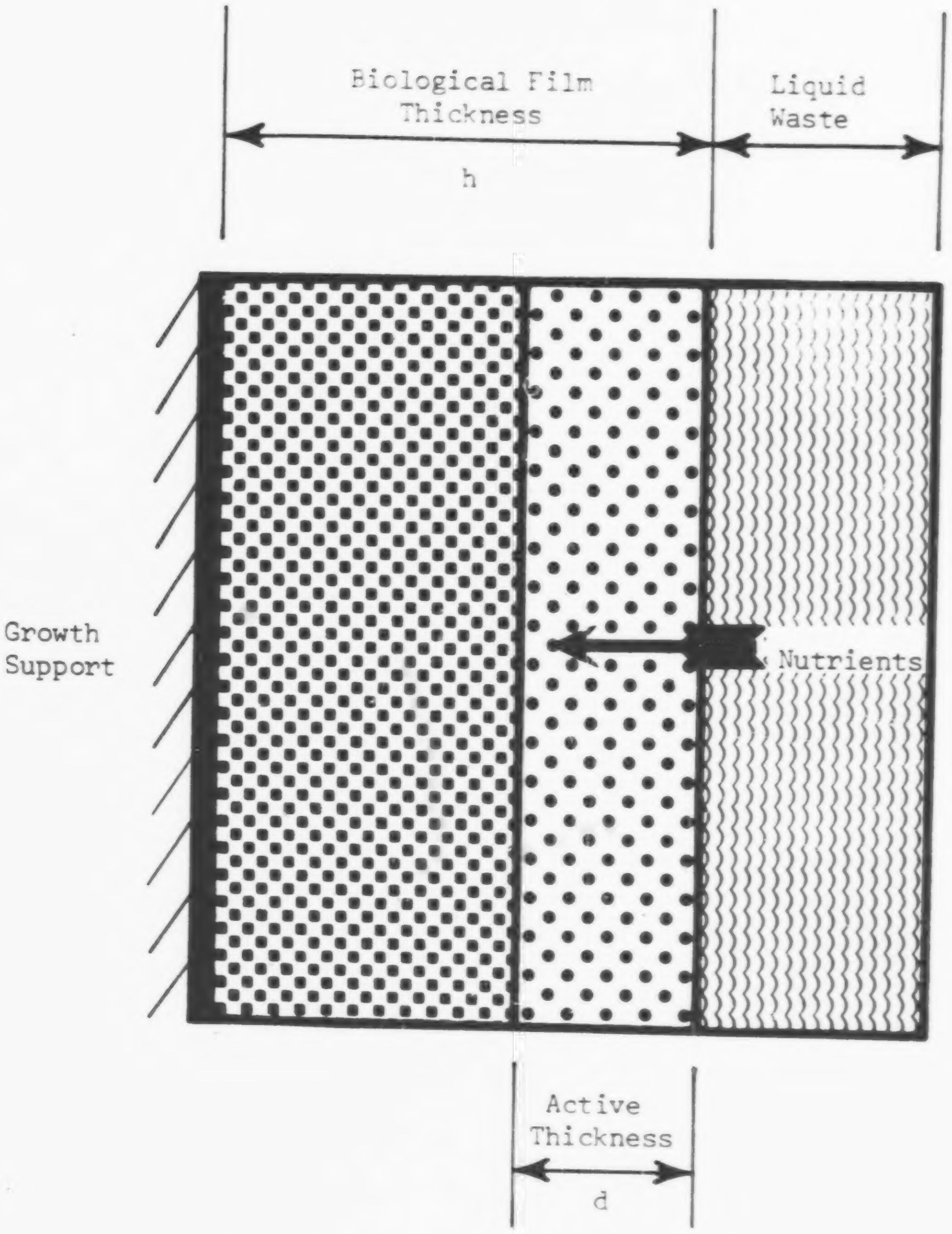
Biological Film
Thickness
h
Liquid
Waste
Growth
Support
Nutrients
Active
Thickness
d

FIGURE 2. BIOLOGICAL FILM SECTION

the plug-flow trickling filter shown in Figure 3 which has a liquid inflow, F and an initial substrate concentration, S_0. The flow remains constant while the substrate concentration decreases with depth, until a concentration S_e is reached at the bottom of the filter. The steady-state substrate balance across the differential element dZ is then

$$\text{Input} = \text{Output} + \text{Disappearance by Reaction} \qquad (40)$$

Since the disappearance of substrate is due to adsorption and subsequent oxidation by the biological film, Equation 40 becomes

$$\text{Input} = \text{Output} + \text{Microbial Utilization} \qquad (41)$$

The substrate input to the differential volume dV is equal to the flow F times the substrate concentration (S + dS). In a similar manner, the output is equal to the flow F times the substrate concentration S. The microbial utilization is equal to the rate of change in the mass of substrate dM_s/dt so the substrate balance reduces to

$$F\,(S + dS) = FS + [dM_s/dt]_{\text{utilization}} \qquad (42)$$

From the work of Monod (3) it can be shown that the decrease in mass of substrate resulting from a corresponding growth of organisms is related by the expression

$$-\,[dM_s/dt] = \frac{1}{Y}\,[dM_o/dt] \qquad (43)$$

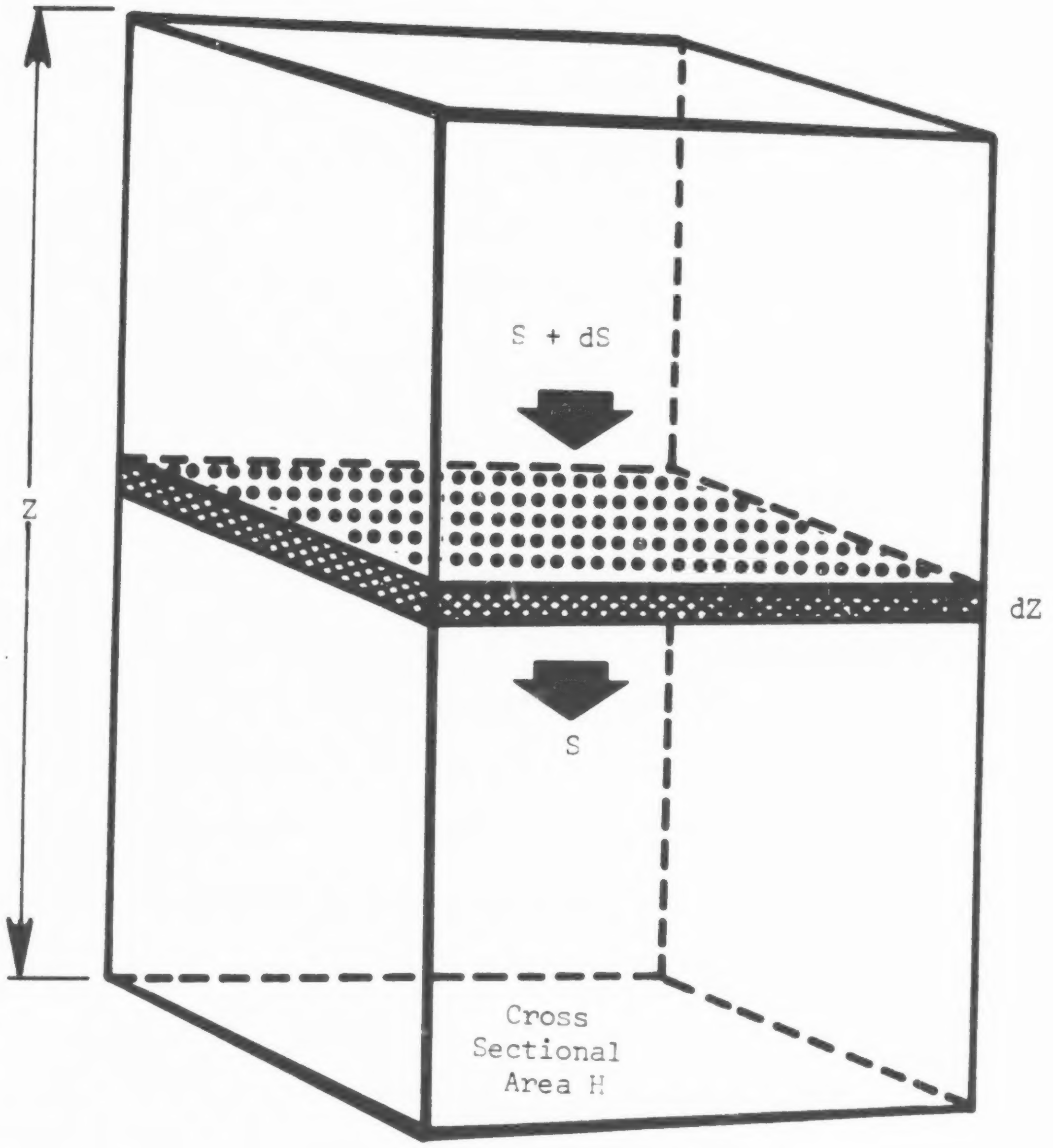
S + dS
Z
dZ
S
Cross
Sectional
Area H

FIGURE 3. SUBSTRATE BALANCE FOR A PLUG-FLOW REACTOR

where M_s = mass of substrate (M)

M_o = active mass of organisms (M)

Y = apparent yield constant (mass of organisms produced/ mass of substrate used).

In addition, the change in organism mass per unit time may be expressed as

$$[dM_o/dt] = \mu M_o \tag{44}$$

where μ = the specific growth rate (T^{-1}).

The substrate balance then becomes

$$F\ (S+dS) = FS - \frac{\mu}{Y} M_o \tag{45}$$

The organisms of interest in this instance are not the total mass, but the active mass of film on the outer surface. This active mass may be expressed as the product of the differential depth dZ, the cross sectional area H, the specific surface area of the filter media "a", the unit mass of the biological film X, and the thickness of the active layer d. Mathematically this is given as

$$M_o = (a)(X)(d)(H)dZ \tag{46}$$

When this expression is substituted in Equation 45 one obtains

$$F\ (S+dS) = FS - \frac{\mu}{Y}\ (a)(X)(d)(H)dZ \tag{47}$$

The resulting expression describing the rate of substrate removal with depth is

$$-\frac{dS}{dZ} = \frac{\mu}{Y}\frac{(a)(X)(d)(H)}{F} \qquad (48)$$

where μ = the specific growth rate constant (T^{-1})

Y = the yield coefficient

S = concentration of the essential nutrient (M/L^3)

a = specific surface area of filter media (L^2/L^3)

X = the unit mass of the microbrial film on a dry basis (M/L^3)

d = the thickness of the active microbial film (L)

H = the cross sectional area of the trickling filter (L^2)

F = the hydraulic flow rate (L^3/T).

Furthermore, the specific growth rate μ may be related to the substrate concentration by the Monod (3) expression

$$\mu = \mu_{max}\left(\frac{S}{K_g + S}\right) \qquad (49)$$

and the substrate removal equation is then

$$\frac{dS}{dZ} = -\frac{\mu_{max}}{FY}(a)(X)(d)(H)\left(\frac{S}{K_g + S}\right) \qquad (50)$$

Before Equation 50 can be integrated to give an expression for the total removal by a trickling filter, it must be determined whether the terms in the equation represent variable or constant values. The cross sectional area of the filter is constant, so it presents no problem.

In most cases the area:volume ratio of the filter media would also be constant. In the event a non-uniform media were used, the area:volume ratio could be expressed as a function of depth, and Equation 50 could then be integrated. If the filter media were applied in layers of uniform area:volume ratios then each layer could be treated as an individual filter and the removal through each layer added to obtain the total removal. At steady state conditions the flow, F would also be constant. The yield constant Y, the concentration of the organisms in the film X, and the maximum growth rate constant are functions of the environmental conditions, the type of substrate, and the organisms which comprise the active film so these should also be constant for a given operating condition. The saturation constant K_g should also be constant for a given flow rate and environmental conditions. The depth of the active film d requires further consideration and investigation, but if, as a first approximation, d is assumed to be constant the total removal through a plug flow filter at steady-state is

$$\int_{S_o}^{S_e} \frac{K_g + S}{S} dS = \int_o^Z - \frac{(\mu_{max})(a)(d)(H)(X)}{FY} dZ \qquad (51)$$

or

$$K_g \ln (S_o/S_e) + (S_o - S_e) = \frac{(\mu_{max})(a)(d)(H)(X)}{FY} Z \qquad (52)$$

In the derivation of Equation 52 no distinction was made between the substrate used for growth and that required for maintenance of the existing population. However, it should be clearly understood that the substrate removal due to maintenance was not neglected, but was incorporated in the apparent growth yield, Y. Using relations presented by Pirt (97) and data by Washington (98) it is shown in Appendix 2 that the apparent yield Y is essentially constant over the region of interest and provides a valid parameter for most engineering applications.

COMPLETELY MIXED, FIXED FILM MODEL

Unfortunately, the number and types of measurements required to test the validity of any equation derived for the plug-flow model of the trickling filter process are difficult and numerous. A more promising approach, for the purpose of constructing a theoretical model of biological films, is the use of completely mixed reactors. By using very low residence times the suspended organisms will be washed out as described by Herbert (99), and any oxygen or substrate utilization must therefore be due to the attached microbial films. The activity of these attached films may then be studied in the more uniform environment of a completely mixed reactor.

The equations derived throughout the remainder of this chapter are based on the following assumptions:

1. Complete mixing is achieved in the liquid phase.
2. Substrate utilization due to sources other than the attached microbial film is small and may be neglected.
3. The apparent yield remains essentially constant.
4. Removal is described by a saturation function which incorporates the effects of diffusion and growth rate.

Substrate Balance

Consider the annular reactor in Figure 4 in which the attached microorganisms utilize oxygen and nutrients from the completely mixed, liquid phase. Substrate is provided at a flow rate F and a concentration.

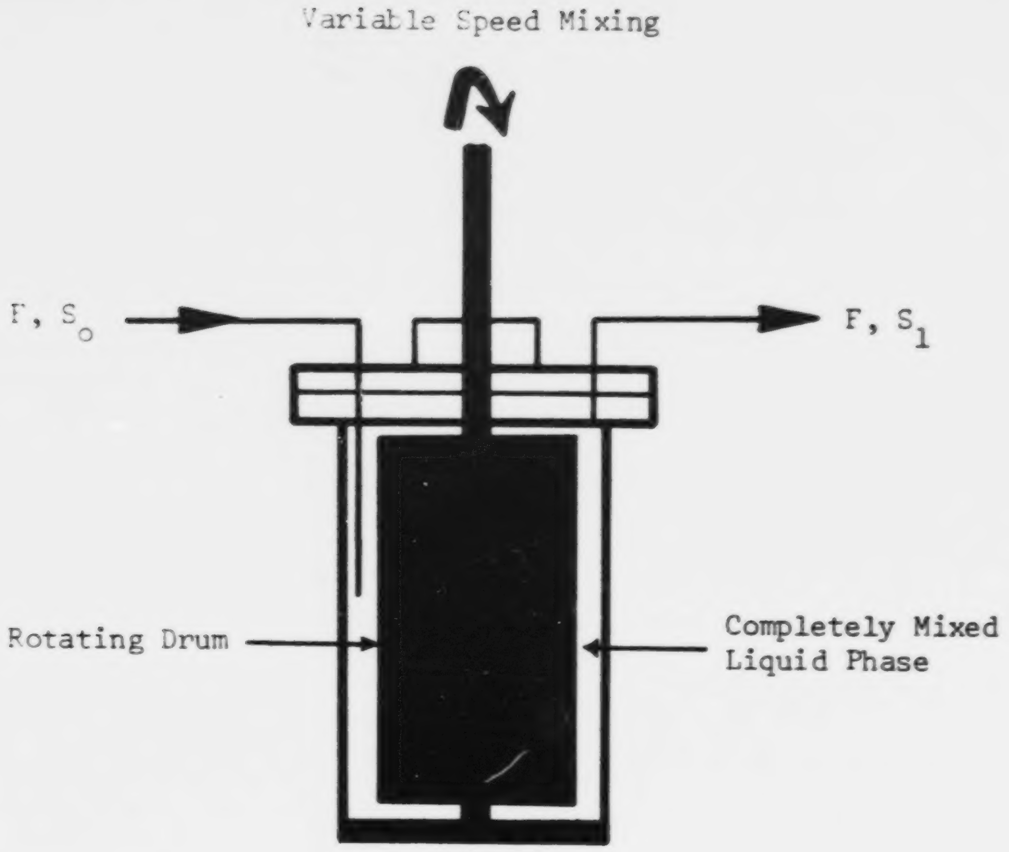
Variable Speed Mixing
F, S_o
F, S_1
Rotating Drum
Completely Mixed
Liquid Phase

FIGURE 4. SUBSTRATE BALANCE FOR AN ANNULAR REACTOR

S_0. The substrate concentration in the reactor and in the effluent are the same and equal to S_1. The decrease in nutrient concentration is due to utilization by the attached microbial films. The mass of film responsible for this utilization is equal to the product of the surface area A, the specific mass of the biological film X, and the depth of active organisms. During periods of film accumulation, the actual film thickness h is less than the active thickness d, and the active mass of film is equal to

$$M_0 = (A)(h)(X) \tag{53}$$

When the actual film thickness h is equal to or greater than the active film thickness d, the active mass is constant and equal to

$$M_0 = (A)(X)(d) \tag{54}$$

Substrate removal and organism growth may be related through the substrate balance

$$\text{Change} = \text{Input} - \text{Output} - \text{Utilization} \tag{55}$$

or

$$[dM_s/dt]_{net} = FS_0 - FS_1 - [dM_s/dt]_{utilization} \tag{56}$$

By substituting the relations

$$-[dM_s/dt] = \frac{1}{Y}[dM_0/dt] \tag{43}$$

and

$$[dM_0/dt] = \mu M_0 \tag{44}$$

into Equation 56, the expression for substrate removal from a completely mixed, fixed film reactor then becomes

$$[dM_s/dt]_{net} = FS_0 - FS_1 - \frac{\mu}{Y} M_0 \tag{57}$$

For the special case where the actual film thickness h is less than the active thickness d, Equation 57 may be written as

$$[dM_s/dt]_{net} = FS_0 - FS_1 - \frac{\mu}{Y} (A)(X)(h) \tag{58}$$

Film accumulation continues until the actual thickness h equals or exceeds the active thickness d. Then the active mass of organisms becomes constant and steady-state is reached with respect to substrate utilization. For this condition

$$[dM_s/dt]_{net} = 0 \tag{59}$$

and Equation 58 reduces to

$$F\ (S_0 - S_1) = \frac{\mu}{Y} (A)(X)(d) \tag{60}$$

When the Monod expression

$$\mu = \mu_{max}\left[\frac{S}{K_g + S}\right] \tag{49}$$

is substituted for the specific growth rate the resulting equation is

$$F\ (S_0 - S_1) = \frac{\mu_{max}}{Y}\,(A)(X)(d)\left[\frac{S_1}{K_g + S_1}\right] \tag{61}$$

Equation 61 expresses the substrate removal from a single stage, completely mixed, fixed-film reactor for varying substrate concentrations. No distinction has been made between substrate used for growth and that required for maintenance for reasons previously cited.

COMPLETELY MIXED, FIXED FILM REACTORS IN SERIES

Before attempting to approximate the trickling filter with completely mixed, annular reactors in series it was desirable to compare the theoretical models of the two systems.

The use of reactors in series to approximate the trickling filters has several obvious advantages. First, this method provides the ease of sampling previously described for the completely mixed, fixed-film annular reactors. Second, flow conditions which range from complete mixing to almost ideal plug flow may be obtained by varying the number of reactors in series. This latter point is significant since flow through packed towers may deviate significantly from ideal plug flow. This fact was reported by Danckwerts (100) and has been verified on actual trickling filters by others (34, 101). The use of multistage reactors to approximate plug flow has been described by Herbert (99) and Powell and Lowe (102) for suspended biological systems and by Greenhalgh, Johnson and Nott (103) for chemical systems. The above studies showed that a plug-flow reactor may be approximated by completely mixed vessels in series. However, a series reactor model was required to ascertain the number of vessels needed to obtain a reasonable approximation of the plug flow model.

In addition to the assumptions specified for the single stage, fixed-film reactor, it must also be assumed that no major shift occurs in organism characteristics in the various reactors for the development of a multistage model.

Substrate Balance

The substrate removal through a series of completely mixed, fixed-film reactors as shown in Figure 5 would be additive so that

$$R = R_1 + R_2 + R_3 \ldots + R_{n-1} + R_n \qquad (62)$$

where R = the total substrate removal through the series of reactors

$R_1, R_2, R_3, \ldots, R_{n-1}, R_n$ = the removal through each individual reactor from reactor 1 through n

n = number of reactors in series.

Since the removal through a single reactor is

$$F\ (S_0 - S_1) = \frac{\mu_{max}}{Y}(A)(X)(d)\left[\frac{S_1}{K_s + S_1}\right] \qquad (61)$$

the removal through a series of reactors would be

$$F\ (S_0 - S_n) = \sum_{o}^{n} \frac{\mu_{max}}{Y}\left[\frac{S}{K_g + S}\right](A)(X)(d) \qquad (63)$$

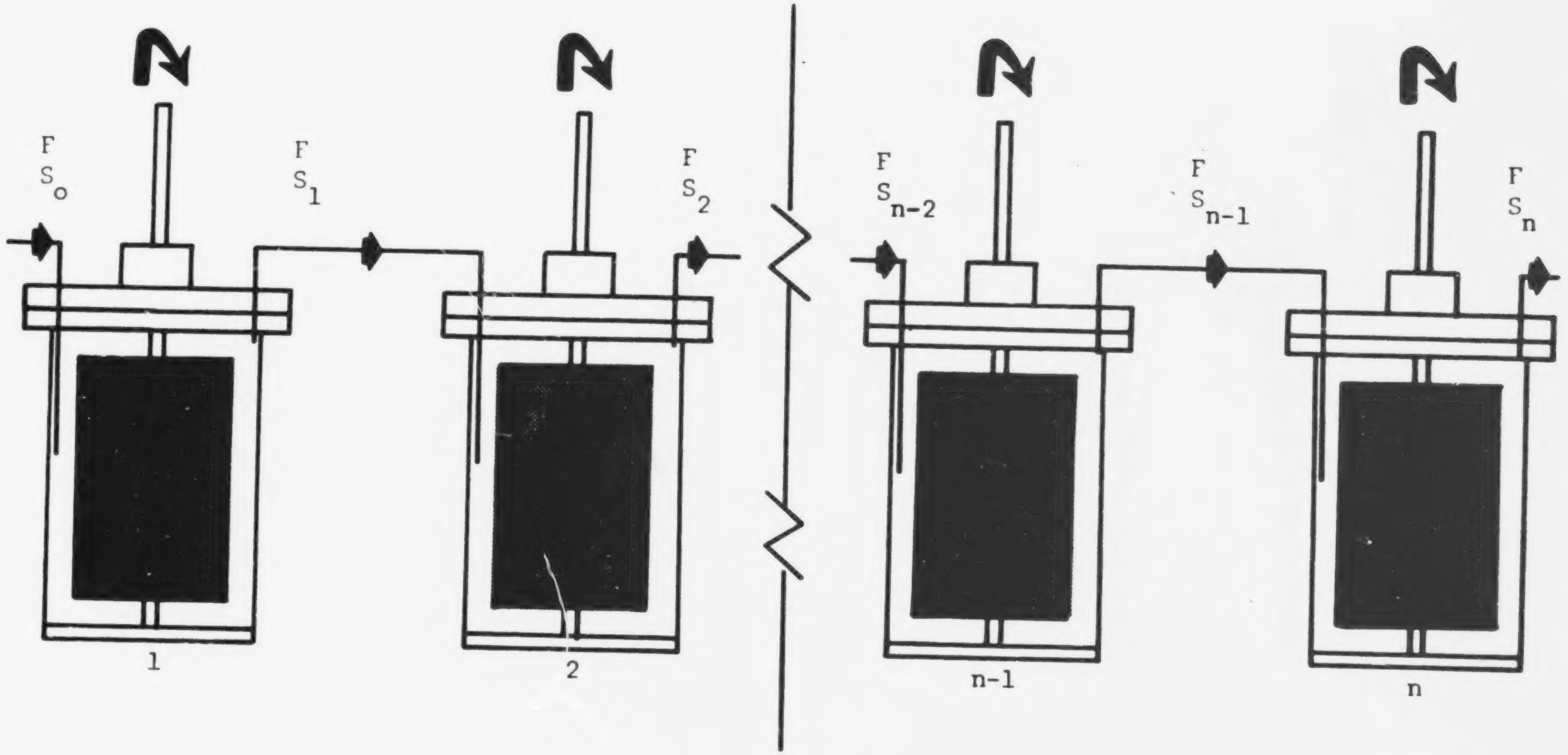
F
S_o
F
S_1
F
S_2
F
S_{n-2}
F
S_{n-1}
F
S_n
1
2
n-1
n

FIGURE 5. SUBSTRATE BALANCE FOR A SERIES OF "n" ANNULAR REACTORS

When the same soluble substrate controls the growth rate in each reactor, which is the only condition of interest here, the maximum specific growth rate constant and yield would not be expected to vary. For identical reactors the area would also be constant, but it must be assumed that the specific organism mass X would be unchanged. For these conditions the removal through the reactors in series would be

$$F(S_0-S_n) = \frac{\mu_{max}}{Y}(A)(X)\sum_{o}^{n}\frac{Sd}{K_g + S} \tag{64}$$

If, as a first approximation, the active thickness d is assumed to be essentially constant then the removal is

$$F\ (S_0-S_n) = \frac{\mu_{max}}{Y}(A)(X)(d)\sum_{o}^{n}\frac{S}{K_g + S} \tag{65}$$

For the special condition where the effluent from the "n"th reactor is very high such that $S_n >> K_g$ the expression

$$\left[\frac{S}{K_g + S}\right] \doteq 1 \tag{66}$$

and the total removal is approximately

$$F\ (S_0-S_n) = \mu_{max}(n)(A)(X)(d) \tag{67}$$

Equation 67 demonstrates the pseudo-zero order of the reaction at high substrate concentrations.

IV. EXPERIMENTAL EQUIPMENT AND ANALYTICAL TECHNIQUES

GENERAL

To test the validity of the theoretical models previously presented and the assumptions upon which they are based, a simple substrate, consisting of glucose and a mineral base, was fed to a mixed population of microorganisms attached to the walls of completely mixed reactors. While the major objective was to determine the rate of substrate utilization and the resulting organism growth, numerous and frequent measurements were also required to insure that such parameters as pH, mixing speed and temperature remained constant.

To obtain the desired data an automated reactor system was employed. Automated or rapid analytical techniques were also necessary in order to avoid sample degradation and to analyze the numerous samples collected. This was particularly valuable during transient studies when large quantities of data had to be analyzed over a short period of time.

EXPERIMENTAL EQUIPMENT AND GENERAL OPERATION

The Annular Reactor

To facilitate the theoretical investigation of biological film kinetics, six annular reactors as shown in Figure 6 and described in Table 1 were designed and built. An annular reactor consisted of an enclosed outer vessel which housed a rotating drum (Figure 7) to

NOT REPRODUCIBLE

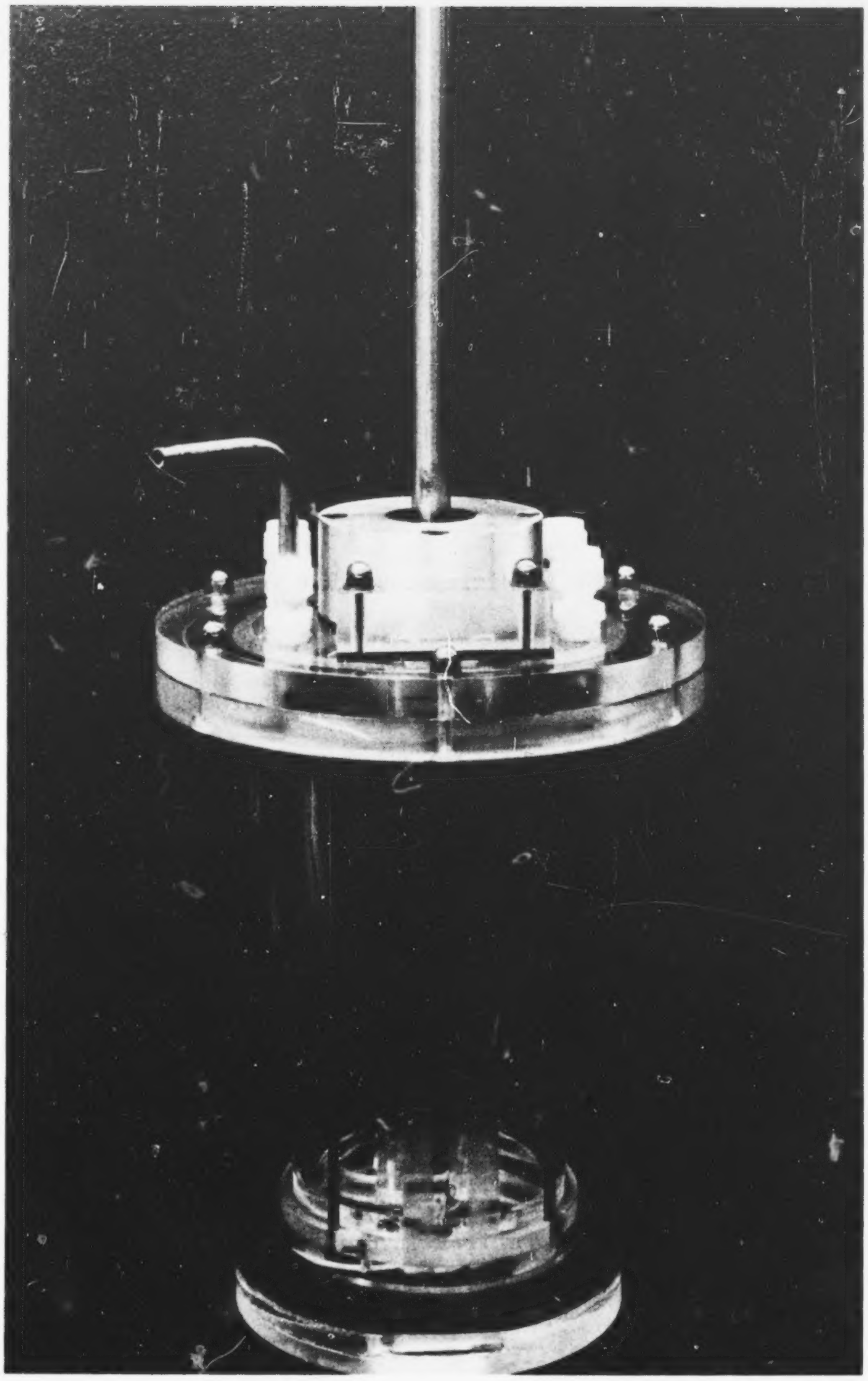

FIGURE 6. ANNULAR REACTOR

NOT REPRODUCIBLE

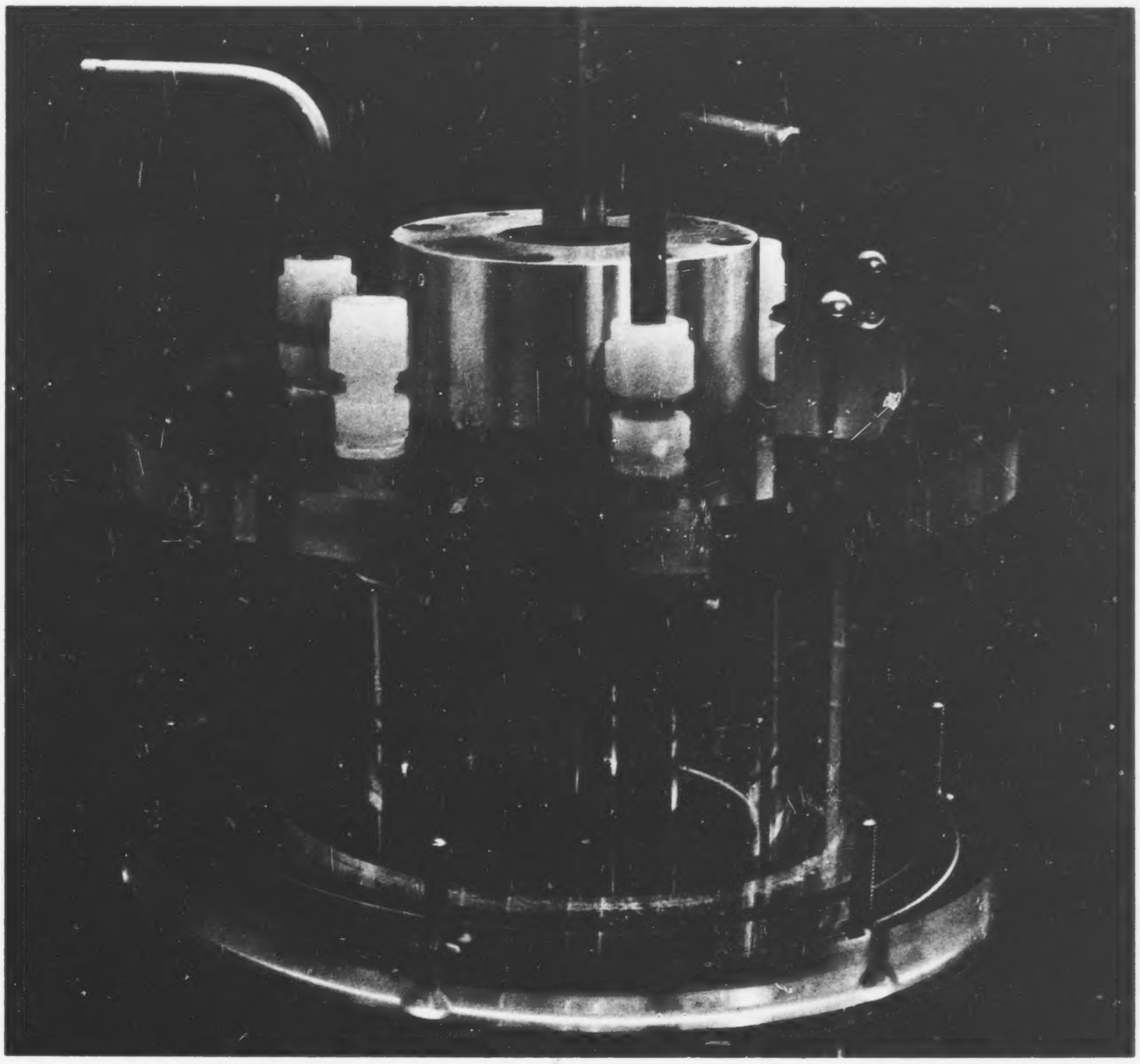

FIGURE 7. PARTING DISASSEMBLY OF AN ANNULAR REALTOR

TABLE 1

DIMENSIONS OF ANNULAR REACTORS

Overall Dimensions	
Outside Diameter	5 inches
Reactor Height	9 inches
Total Volume	1000 ml.
Total Internal Surface Area (Includes internal feed and sample lines)	2000 sq. cm.
Inside Dimensions	
Inside Diameter	4.50 inches
Inside Height	8.00 inches
Drum Dimensions	
Outside Diameter	3.50 inches
Total Height	7.25 inches
Draft Tube Diameter (O.D.)	0.50 inches
Draft Tube Height	6.875 inches
Height of Impeller Assembly (Includes 0.125 in cover)	0.375 inches
Slide Dimensions	
Total Length	8.75 inches
Internal Exposed Length	8.00 inches
Width of Exposed Face	1.125 inches
Thickness (Back machined to produce 2.25 inch radius and thereby provide integral wall fit)	0.063 inches
Exposed Surface Area	9 sq. inches

promote mixing, decrease the liquid volume and increase the available surface for biological films. Mixing was enhanced by the pumping action of four draft tubes and an impeller assembly (Figure 8) which were built into the rotating drum. A removable slide (Figure 9), machined to provide an integral fit with the side wall, was also installed in order to determine the thickness and specific mass of the biological film. The entire reactor volume of one liter was filled with liquid so the internal surface of 2000 cm^2 was also the area of biological growth.

Reactor Arrangements and Mixing Power

Two water baths, with a capacity of three reactors each, were constructed on opposite sides of a laboratory support frame as shown in Figure 10. Mixing power for the rotating drum was provided by individual GT-21 laboratory motors which provided mixing speeds from 0 to approximately 330 rpm at 6 inch-lbs of torque. The GT-21 consists of a 1/40th h.p. Bodine motor and a Heller variable speed controller. The motor and reactor arrangement is also shown in Figure 10.

Substrate Dilution System

Due to the high flow rates employed it was impractical to store substrate at the desired influent concentration. Therefore, a substrate dilution system as shown in Figure 11 was employed. Substrate was initially prepared as shown in Table 2 to produce a concentrated mixture. Twice each day this mixture was manually diluted with distilled water to a 10 liter volume having a concentration approximately 35 times greater than that desired in the reactor influent. This feed substrate was stored in a 3 gallon Nalgene wide mouth carboy and continuously mixed by an 1800 rpm mixing motor which was mounted

NOT REPRODUCIBLE

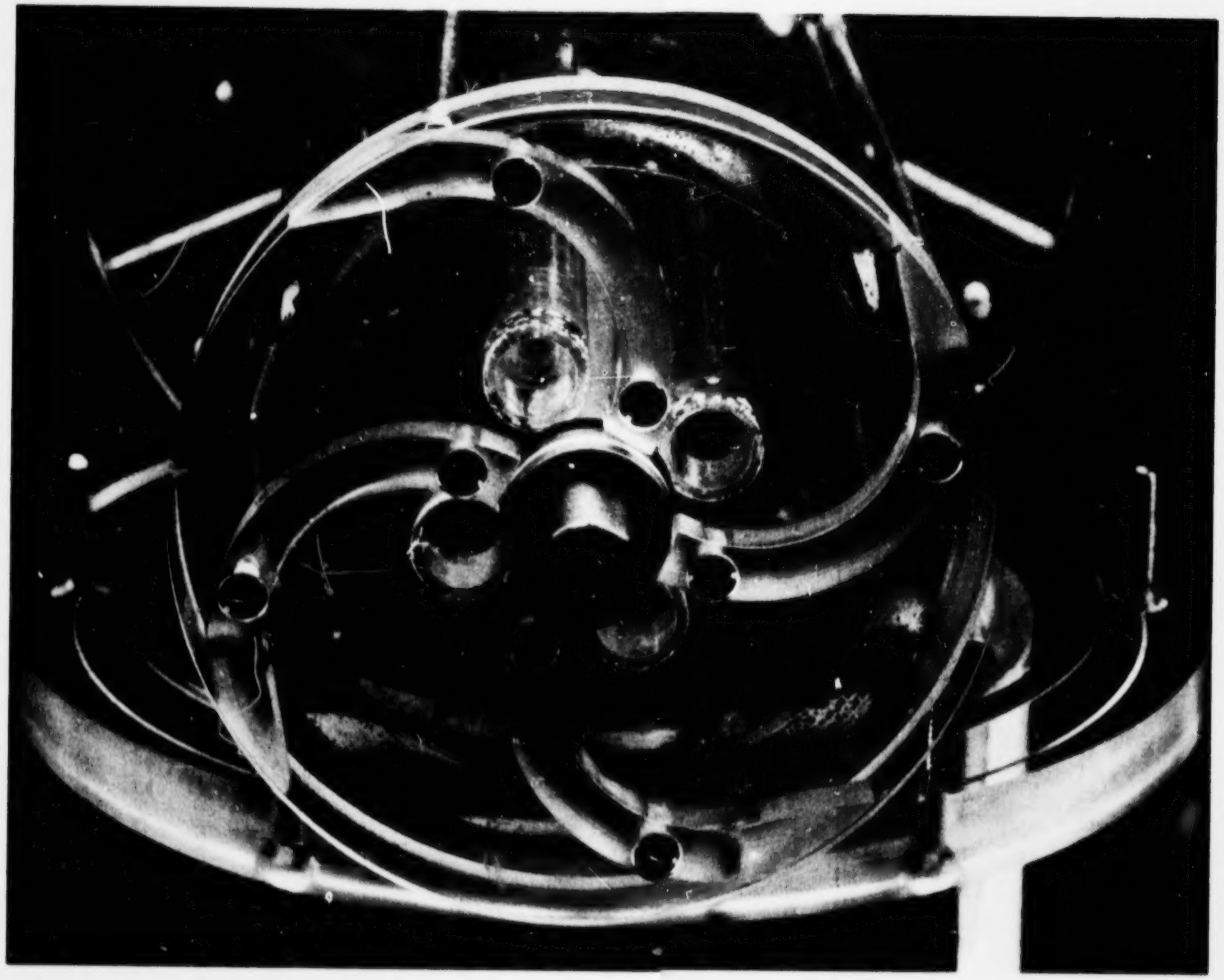

FIGURE 8. DRAFT TUBE AND IMPELLER ASSEMBLY

NOT REPRODUCIBLE

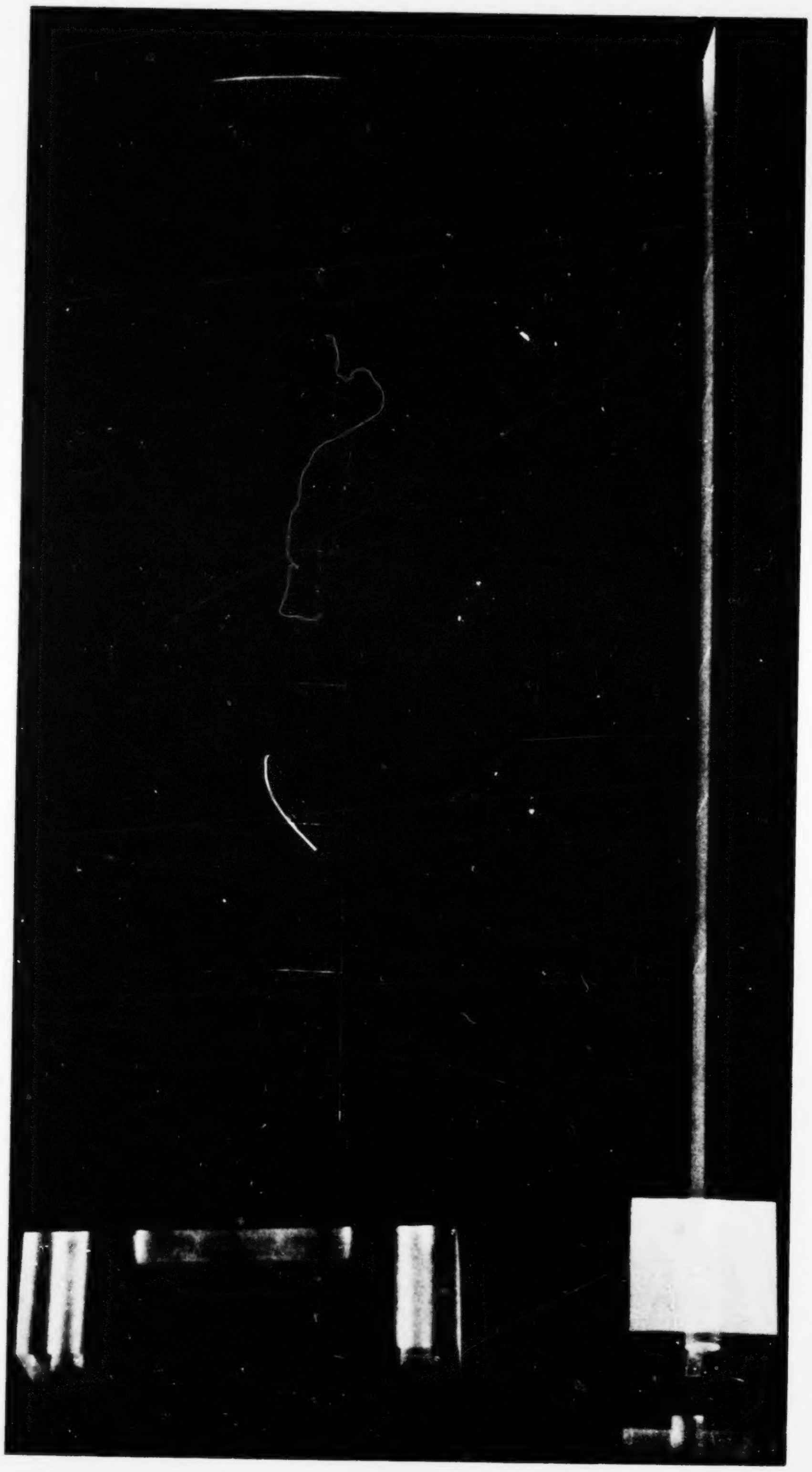

FIGURE 9. REMOVABLE SLIDE

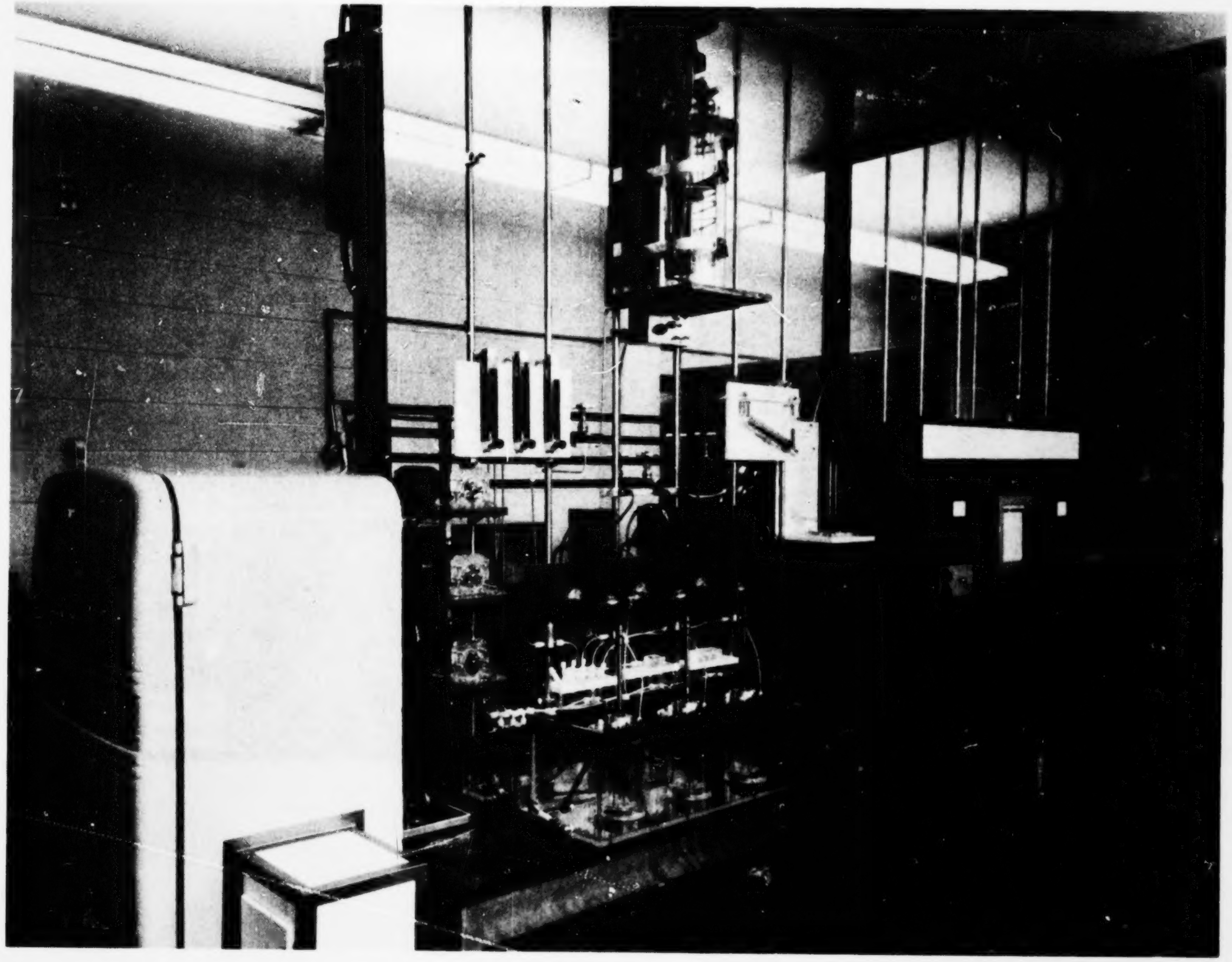

FIGURE 10. EXPERIMENTAL APPARATUS

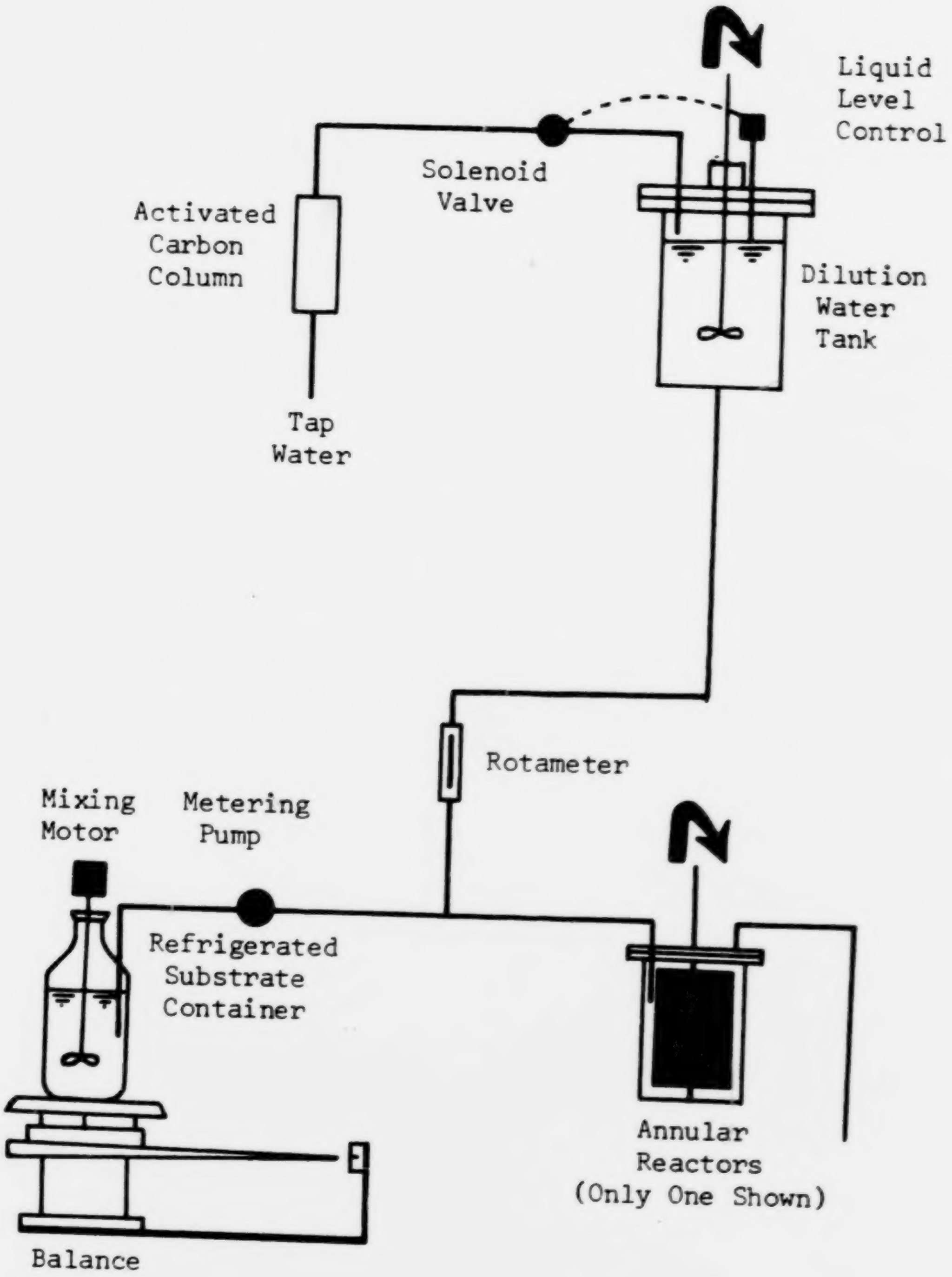
Liquid Level Control
Solenoid Valve
Activated Carbon Column
Dilution Water Tank
Tap Water
Rotameter
Mixing Motor
Metering Pump
Refrigerated Substrate Container
Annular Reactors (Only One Shown)
Balance

FIGURE 11. SCHEMATIC DIAGRAM OF THE SUBSTRATE DILUTION SYSTEM

TABLE 2

INITIAL SUBSTRATE COMPOSITION

Trace Salt Solution (Composition shown below)	33.30 ml
KH_2PO_4	13.61 gms
$NaH_2PO_4.H_2O$	8.28 gms
$(NH_4)_2SO_4$	5.28 gms
NH_4Cl	49.20 gms
$CaCl_2$	4.44 gms
$MgCl_2.6H_2O$	8.13 gms
Glucose	144.0 gms
Yeast Extract	3.6 gms

Bring to 1 liter with distilled H_2O.

COMPOSITION OF TRACE SALT SOLUTION

Compound	Mass
$FeCl_3$	38.88
$MnCl_2.4H_2O$	9.48
$ZnCl_2$	6.54
$CuCl_2.2H_2O$	4.10
$CoCl_2.6H_2O$	5.71
$(NH_4)6Mo_7O_{24}.4H_2O$	4.15
$Na_2B_4O_7.10H_2O$	2.29
Na_3Citrate	353.0

Bring to two liters with distilled H_2O.

through the top. One-quarter inch stainless steel feed and sample lines were then inserted through holes in the shoulder of the carboy and the entire container was placed on a 20 kg. Ohus balance in a refrigerator at 5°C. The Ohus balance provided a convenient and reliable method for determining the quantity of feed substrate used.

The desired influent concentration to the reactor was obtained by regulating the speed of the metering pump which moved the feed substrate from the refrigerated container to a point near the reactor influent where it was mixed with buffered dilution water. The dilution water flow was controlled by rotameters as it flowed by gravity from the constant head, dilution tank to the point where it joined the feed substrate. The combined stream then flowed into the reactor where it was discharged in the half-inch annular space. Since the dilution water flow was maintained between 128-132 ml/min and the flow of the feed substrate was only about 4 ml/min, the flow to the reactors was essentially that of the dilution water. Therefore, the substrate concentration could be changed by varying the rate of substrate feed without materially changing the total flow rate. Water in the 10 liter dilution tank was replenished by tap water which passed through an activated carbon column to remove residual chlorine prior to flowing into the tank. A constant head and volume were maintained through the use of a Frost liquid level relay which controlled a two-way solenoid valve that regulated the rate at which tap water entered the dilution tank.

Temperature

The dilution system not only provided a means of achieving high flow rates without excessive storage, but was also vital in the control of temperature. Due to the low retention time in the reactor it was imperative that the influent be maintained at the desired temperature of 25 ± 0.5°C. This was accomplished by regulating the temperature of the dilution water. As shown in Figure 12 water was pumped from the number two water bath through a heat exchanger in the dilution tank and then returned to the number one water bath. A large hose connected the two barhs so water was continuously cycled through the loop. The temperature of the heating baths was regulated by a YSI temperature controller which actuated a heating or cooling device to maintain the desired conditions. Heat was provided by a 500 watt heating coil and cooling was achieved by the addition of cold water. The flow of cold water was controlled by a two-way solenoid valve which was operated by the YSI controller. An overflow system was used to maintain a constant level in the heating baths.

Dissolved Oxygen

Since the entire supply of oxygen to the reactors was dissolved in the influent stream, the dilution system also provided a means of controlling the oxygen content of the reactors. Oxygen and nitrogen were metered and blended as they flowed from storage tanks to the dilution tank where the mixture was discharged below a high speed impeller which provided complete mixing. A Matheson Gas Proportioner was used to regulate the quantity and composition of the gas being supplied to the tank. The entire system was capable of being operated under pressure so dissolved oxygen concentrations in excess of 50 mg/l could be obtained in the dilution water. A schematic diagram of the system is shown in Figure 13.

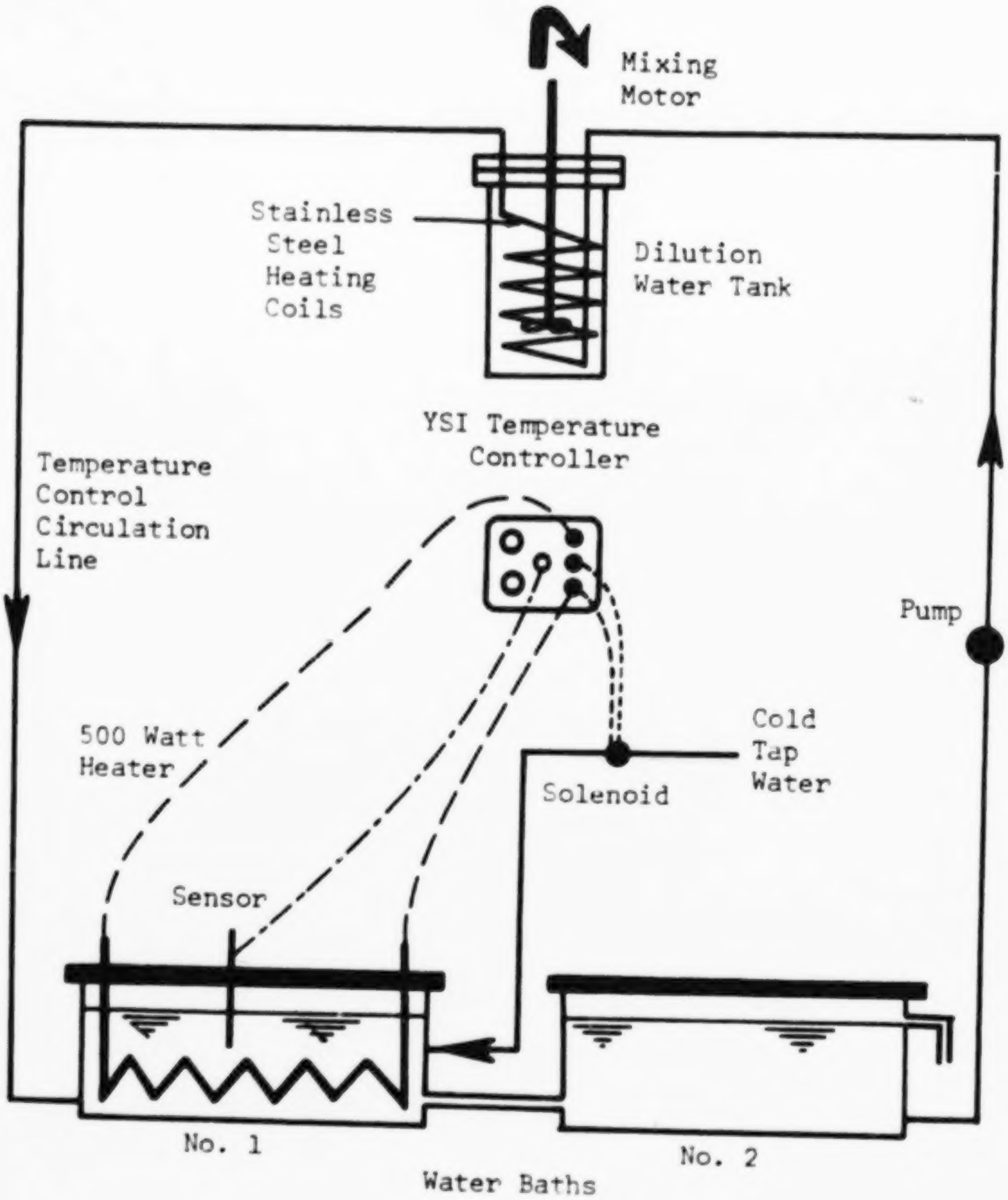
Mixing
Motor
Stainless
Steel
Heating
Coils
Dilution
Water Tank
YSI Temperature
Controller
Temperature
Control
Circulation
Line
Pump
500 Watt
Heater
Cold
Tap
Water
Solenoid
Sensor
No. 1
No. 2
Water Baths

FIGURE 12. SCHEMATIC DIAGRAM OF THE TEMPERATURE CONTROL SYSTEM

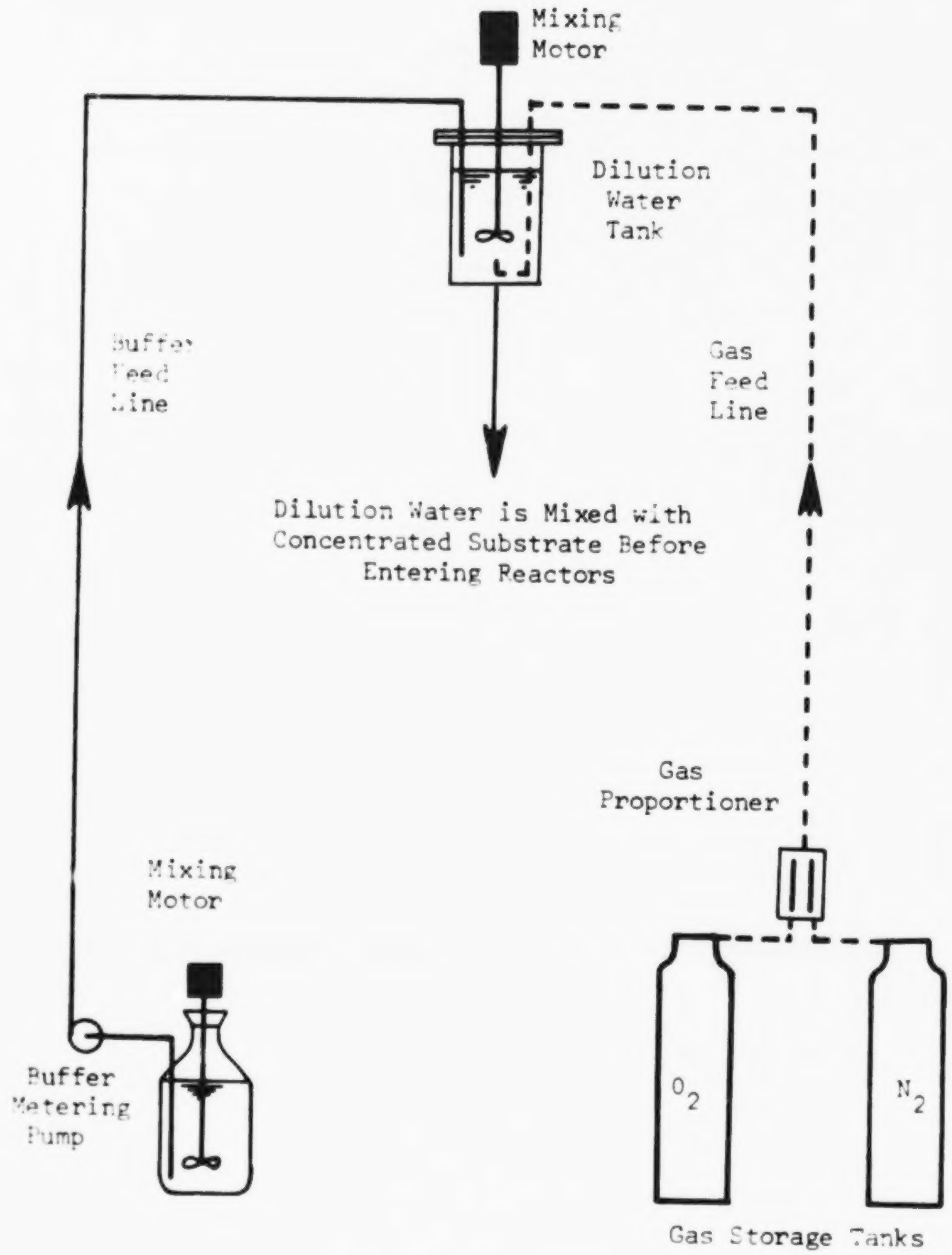
Mixing Motor
Dilution Water Tank
Buffer Feed Line
Gas Feed Line
Dilution Water is Mixed with Concentrated Substrate Before Entering Reactors
Gas Proportioner
Mixing Motor
Buffer Metering Pump
O_2
N_2
Gas Storage Tanks

FIGURE 13. SCHEMATIC DIAGRAM OF THE DISSOLVED OXYGEN AND pH CONTROL SYSTEM

pH

Also shown in Figure 13 is the pH control system which, like temperature and dissolved oxygen, was dependent on the dilution water. A 1.5 M phosphate buffer was pumped from a continuously mixed storage container into the dilution tank where it was mixed with the water by the high speed impeller. The flow rate of the constant speed buffer pump was 2.8 ± 0.1 ml/min and the buffer concentration in the reactors was approximately 0.007 molar. This was sufficient to maintain a pH of 6.5 ± 0.1 in the reactors.

Effluent Monitoring System

During the first two phases of this investigation three reactors were operated in parallel and under the same conditions for each test. As shown in Figure 14 the effluent stream was taken from the top of the reactors to prevent gas accumulation and then passed through three-way solenoid valves which normally diverted the flow to waste. A Cramer timer opened the solenoid valves in sequence so the dilution water and effluent from each reactor was diverted past a Beckman Dissolved Oxygen probe and YSI thermistor for a period of 10 minutes during each 40 minute cycle.

The amplified output from the Beckman Dissolved Oxygen Analyzer was fed into a bank of electrical relays. As the timer opened a solenoid valve it also opened the corresponding relay and the data was printed as a selected point on an L & N Multipoint Recorder. The temperature of any stream could be identified through an association with the corresponding dissolved oxygen point. A schematic circuit diagram of the system is shown in Figure 15.

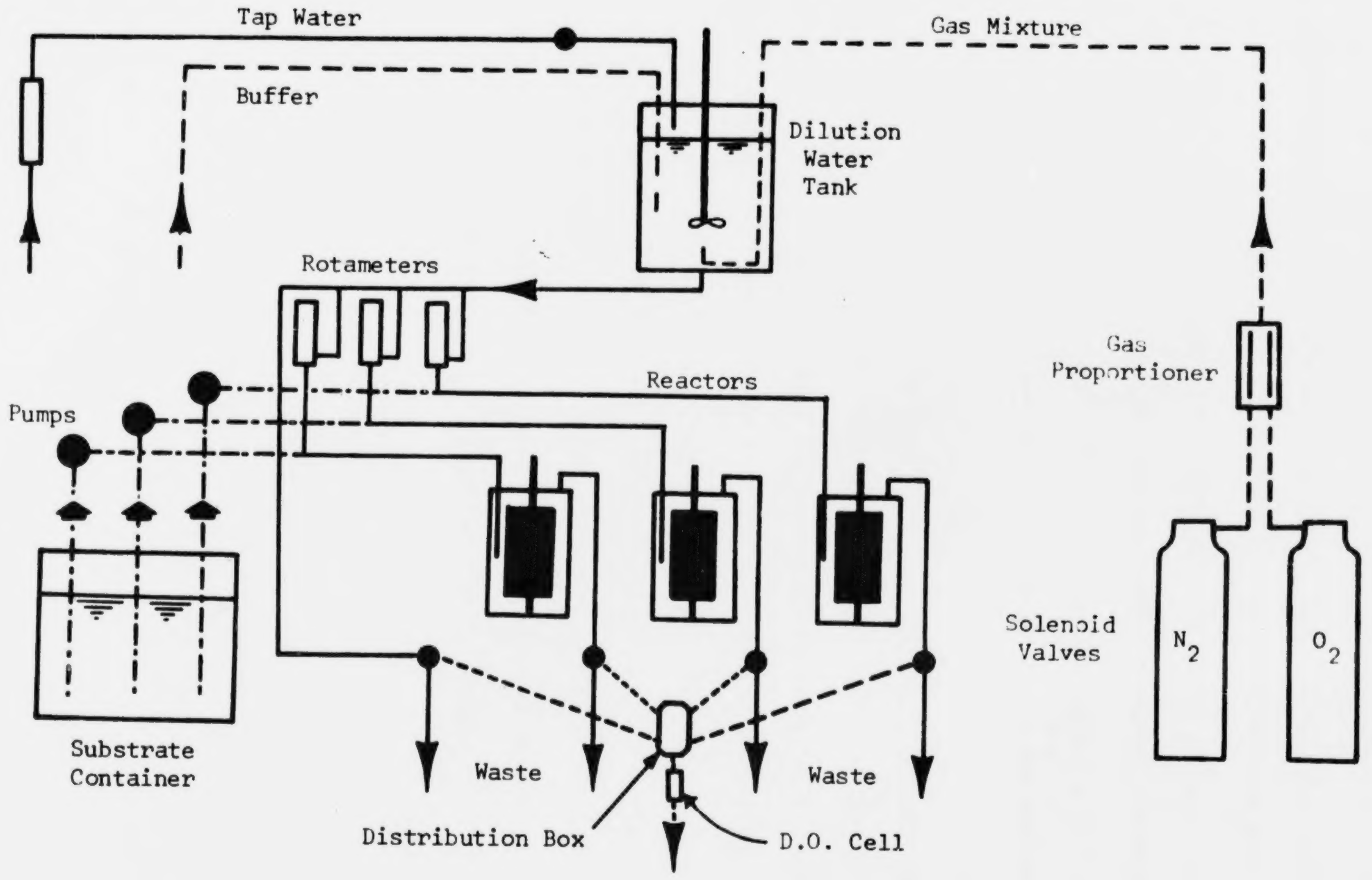
Tap Water
Buffer
Gas Mixture
Dilution Water Tank
Rotameters
Reactors
Pumps
Gas Proportioner
Solenoid Valves
N_2
O_2
Substrate Container
Waste
Waste
Distribution Box
D.O. Cell

FIGURE 14. SCHEMATIC DIAGRAM OF THE EXPERIMENTAL APPARATUS

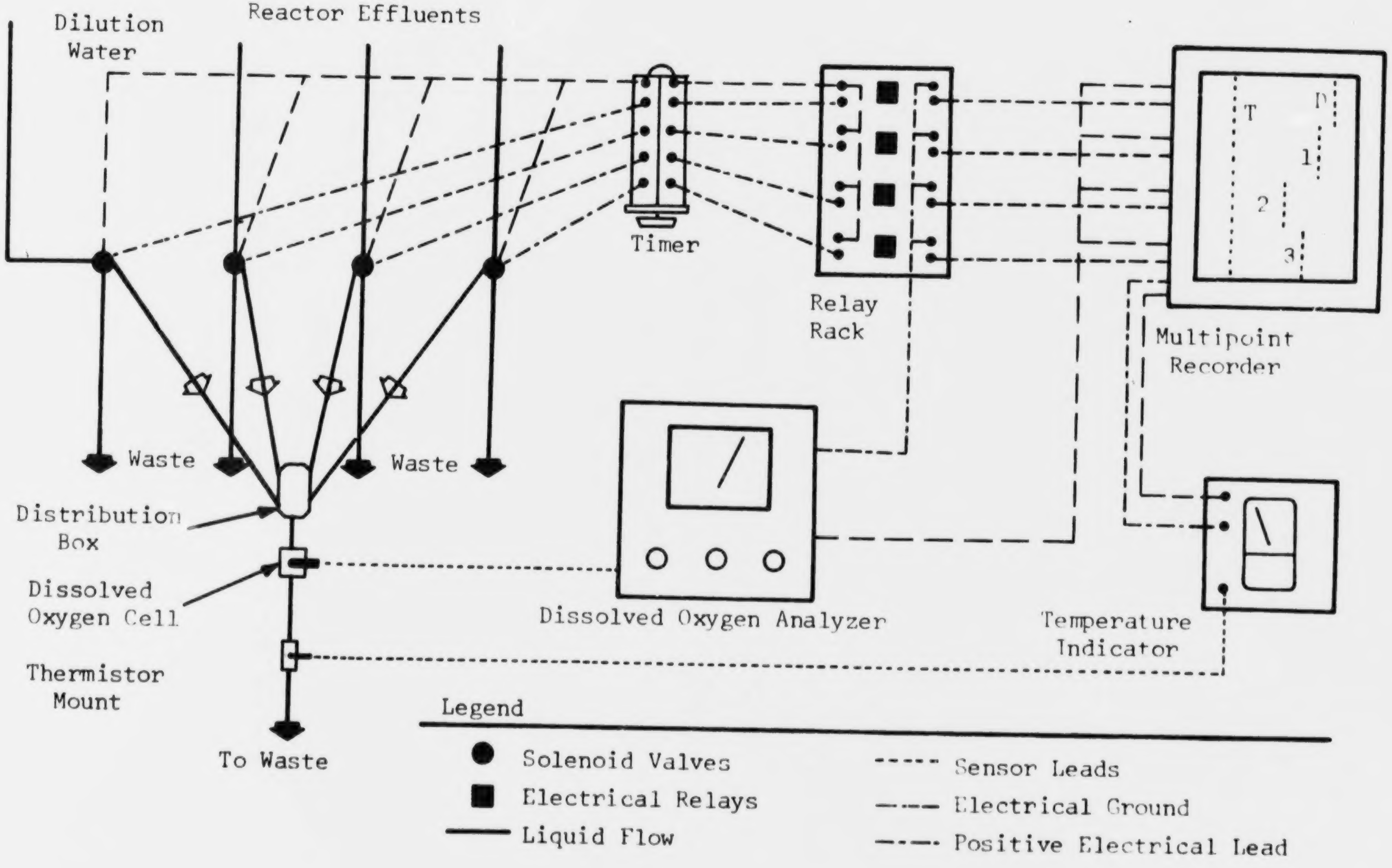
Dilution
Water
Reactor Effluents
Timer
Relay
Rack
T
D
1
2
3
Multipoint
Recorder
Waste
Waste
Distribution
Box
Dissolved
Oxygen Cell
Thermistor
Mount
To Waste
Dissolved Oxygen Analyzer
Temperature
Indicator
Legend
● Solenoid Valves
■ Electrical Relays
Liquid Flow
Sensor Leads
Electrical Ground
Positive Electrical Lead

FIGURE 15. SCHEMATIC DIAGRAM OF THE EFFLUENT MONITORING AND RECORDING CIRCUIT

During Phase III six of the reactors were operated in series and the effluent from the sixth reactor continuously flowed past the Beckman D.O. probe and YSI thermister. The signal from the probes was amplified by the Beckman D.O. Analyzer and YSI Indicator and printed on the L&N Multipoint Recorder on a continuous basis. The dissolved oxygen concentration of the other five reactors was determined from manually collected samples.

ANALYTICAL TECHNIQUES

Substrate Determination

Glucose was employed as the carbon source in this investigation as rapid analytical techniques were readily available. A Technicon Auto Analyzer, employing a modification of the potassium ferricyanide-potassium ferrocyanate reduction reaction (104) was used to determine all influent and effluent substrate concentrations in triplicate. To insure the procurement of representative samples a 50 ml volume was collected from each effluent and a 250 ml volume from each influent stream. The larger influent sample was required due to the slight pulsation of the substrate feed pump. The sample lines were always cleared of entrapped liquids by allowing approximately 100 ml to flow to waste prior to sample collection. It was found in preliminary studies that the sloughed biomass in effluent samples gradually coated the dialysis membrane of the Technicon Auto Analyzer. Therefore, suspended solids were removed by filtering the sample through Whatman No. 1 filter paper during the collection process. All collected samples were preserved with 0.1 ml of saturated $HgCl$ per 50 ml of sample and stored under refrigeration at 5°C. Although the $HgCl$ interferred with the test procedure, the error introduced was small and predictable as shown in Appendix 3.

Chemical Oxygen Demand (105, p. 399) tests were also conducted during preliminary studies to insure that glucose provided a representative indication of the organics removed. It was found that the organic removal, as indicated by glucose determinations, was proportional to that determined by COD. Therefore, the latter method of testing provided a duplication of data and was discontinued.

Dissolved Oxygen Determination

The dissolved oxygen concentration was determined primarily by the continuous effluent monitoring system but manually collected samples from each reactor were also run several times daily as a check on the automated system. The probe of the Model 778 Beckman Dissolved Oxygen Analyzer was changed daily and the system calibrated with three samples determined by the Pomeroy-Kirchman-Alsterberg modification of the Winkler Method (105, p. 316). The manually collected samples were run by the above method when concentrations in excess of 15 mg/l were encountered and by the Alsterberg modification of the Winkler Method (105, p. 309) for lower dissolved oxygen concentrations. The influent dissolved oxygen concentration required to maintain aerobic conditions during periods of high substrate input could not be determined by chemical methods and degasification prevented the collection of samples for manometric analysis. Therefore, the influent dissolved oxygen could not be determined upon occasion, but the effluent was monitored on a continuous basis.

Biological Film Thickness

The thickness of the biological film was determined with the stage micrometer of a microscope as reported by Sanders (56). The microscope was focused on the film surface and then on the interface of the biological film and the removable slide. The film thickness was determined from the difference in the two micrometer readings. The reported film thickness was calculated from the readings taken along the slide from top to bottom. The micrometer was calibrated by determining the thickness of transparent objects which ranged in thickness from 1 mil mylar films to 1/64" plastic sheets. The actual thickness of the transparent objects was determined with a precision micrometer. Lines were drawn on opposite sides of the objects at right angles to assist in the calibration method. It was found that each unit on the Wild M20 microscope used in this investigation represented two microns of thickness.

Suspended Solids

Total and volatile suspended solids were determined on each sample in duplicate by the glass fiber filter technique using aliquots of from 250 to 1000 ml. The size of sample was adjusted within this range to obtain at least a 10 mg increase in weight whenever possible. Unfortunately, it was not always possible to obtain the 10 mg increase desired since a one liter sample yielded less than 0.1 mg of suspended solids during early periods of film accumulation. Since these early determinations were not required for the calculation of microbial growth constants, a volume larger than one liter was not used due to the time necessary for sample collections.

As indicated in the procedure in Appendix 3 two filters were used for rigidity and ease of handling. The washed, dried and weighed filter was placed on the vacuum filter apparatus before samples were collected to reduce the possibility of erroneous results due to biological growth or degradation during storage. All weights were determined on a Mettler balance to the nearest hundredth of a mg 35 seconds after the filter was removed from the dessicator.

Cell Composition Determinations

The composition of cellular material, both suspended and attached, was determined in triplicate on the freeze dried samples using the F and M Model 185 Carbon-Hydrogen-Nitrogen Analyzer. The oxygen composition of the cells was taken as the difference between the volatile portion of the sample and the sum of the carbon, nitrogen and hydrogen fractions.

Temperature

The temperature of all streams was determined by a YSI thermistor and recorded on a L&N Model H recorder as previously described in the equipment section. The thermistor was calibrated with a mercury thermometer during each run.

Liquid Flow Systems

All pumps and rotometers were calibrated prior to beginning this investigation and the calibration was checked daily. The method used for both the calibration and daily check was to collect a known liquid volume during a period of time measured with a stop watch.

Mixing Speed

The mixing speed of the rotating drum was determined initially by two methods. A Strobatic stroboscope was used as well as a manual time count. The latter method proved to be more accurate and easier at mixing speeds employed in the study and the stroboscope method was discontinued.

Organism Characteristics

To insure that no major shifts occurred in the characteristic of the microbial population between the various runs, Gram-stains were prepared from each reactor. These were examined microscopically for Gram-stain and morphological features, but organism identification and classification were beyond the scope of this study.

V. MIXING STUDIES

GENERAL

After the analytical techniques were established and the mechanical equipment evaluated, it was necessary to conduct several studies to determine the mixing speed to be used during the investigation. The paramount interest in these studies was the effect of mixing speed on:

1. Residence time distribution of the liquid.
2. Biological film accumulation.
3. Rate of substrate utilization.

Although complete mixing was desired the mixing speed could not be so high that film accumulation would be prevented. It was essential that the total film thickness exceed the "active" or "critical" film thickness as reported by Sanders (56) and investigators in England (55). In addition, the effect of liquid film diffusion was also of interest. Based on work by Swilley et al. (106) it was felt that liquid film diffusion could materially influence the substrate or dissolved oxygen concentration available at the film surface and should be considered. Any change in substrate utilization with mixing would restrict the use of measured biological constants to mixing ranges in which they were determined. It was, therefore, imperative that the effects of mixing be investigated.

RESIDENCE TIME DISTRIBUTION

Procedure

The mixing characteristics were studied at drum rotation speeds from 25 to 200 rpm. A Turner Fluorometer was used to obtain residence time distribution curves. For these studies a single reactor was operated at experimental temperatures and flow rates but the 134 ml/min flow to the reactor was water instead of the synthetic substrate. This modification was instituted to preclude adsorption of dye by the biological film. After experimental flow rates were established, approximately 5 ml of Rhodamine 6 GDN extra dye solution (10 mg/l) was pulse injected into the reactor with a syringe. The syringe was locked into one of the Swagelok sample ports in a position similar to that of the substrate influent line.

The reactor effluent was passed through the continuous flow cell of the Turner Fluorometer and the effluent concentration was continuously recorded. Residence time distribution curves were obtained at 25, 50, 100 and 200 rpm. The curves were converted to a reduced to a reduced form as described by Danckwerts (100) and compared to the curve for ideal mixing. The test conditions and tabular results are given in Appendix 4.

Results and Discussion

The results of the residence time distribution studies clearly showed that the degree of mixing was a function of mixing speed. This is shown in Figures 16 and 17 where C-curves for mixing speeds of 25, 50, 100 and 200 rpm are compared to the ideal mixing curve $e^{-\theta}$, where θ is the ratio of the actual to theoretical residence time. If the area of the residence time distribution curve lying above $e^{-\theta}$ is used

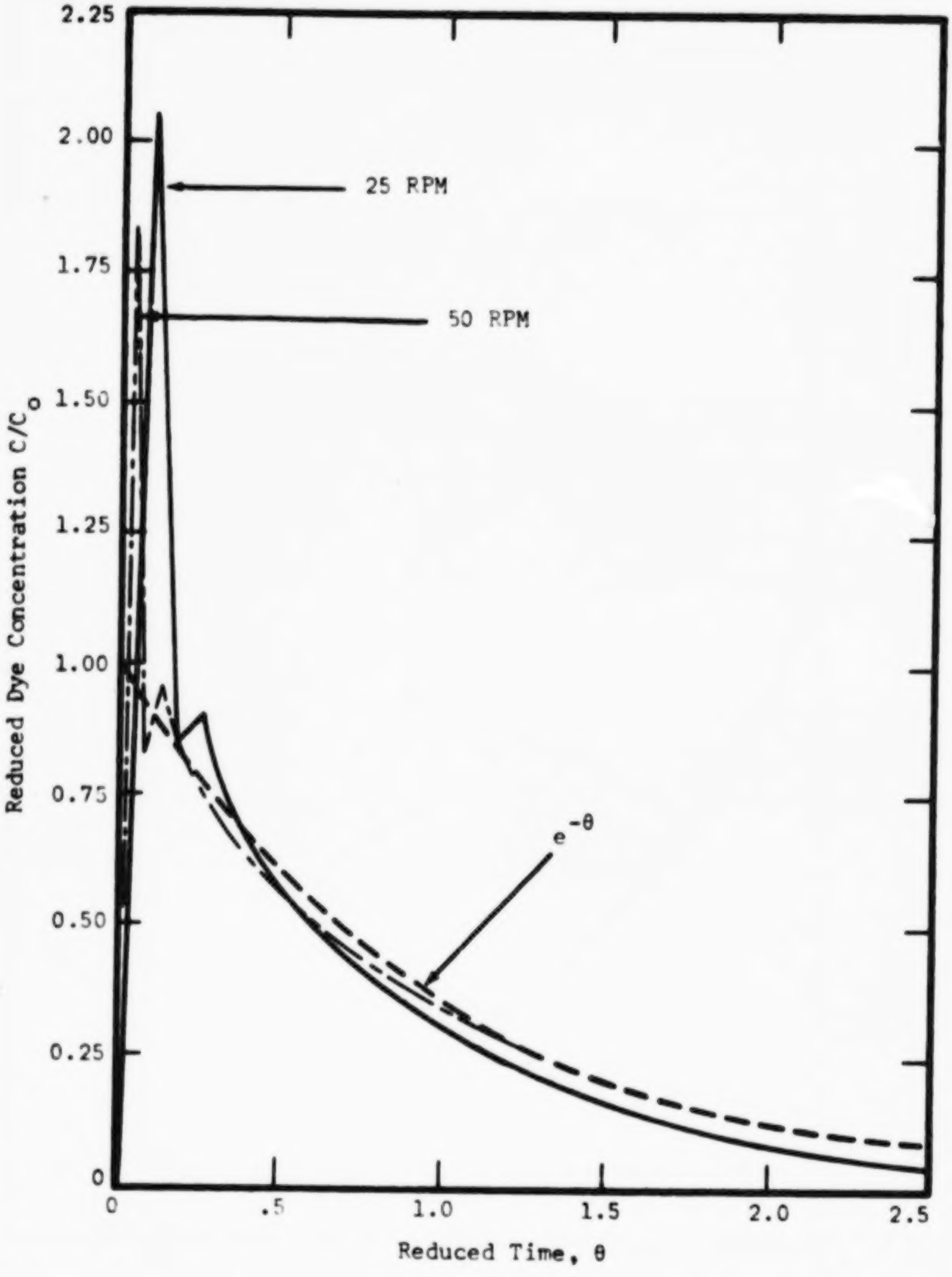

FIGURE 16. RESIDENCE TIME DISTRIBUTION CURVES FOR MIXING SPEEDS OF 25 and 50 RPM

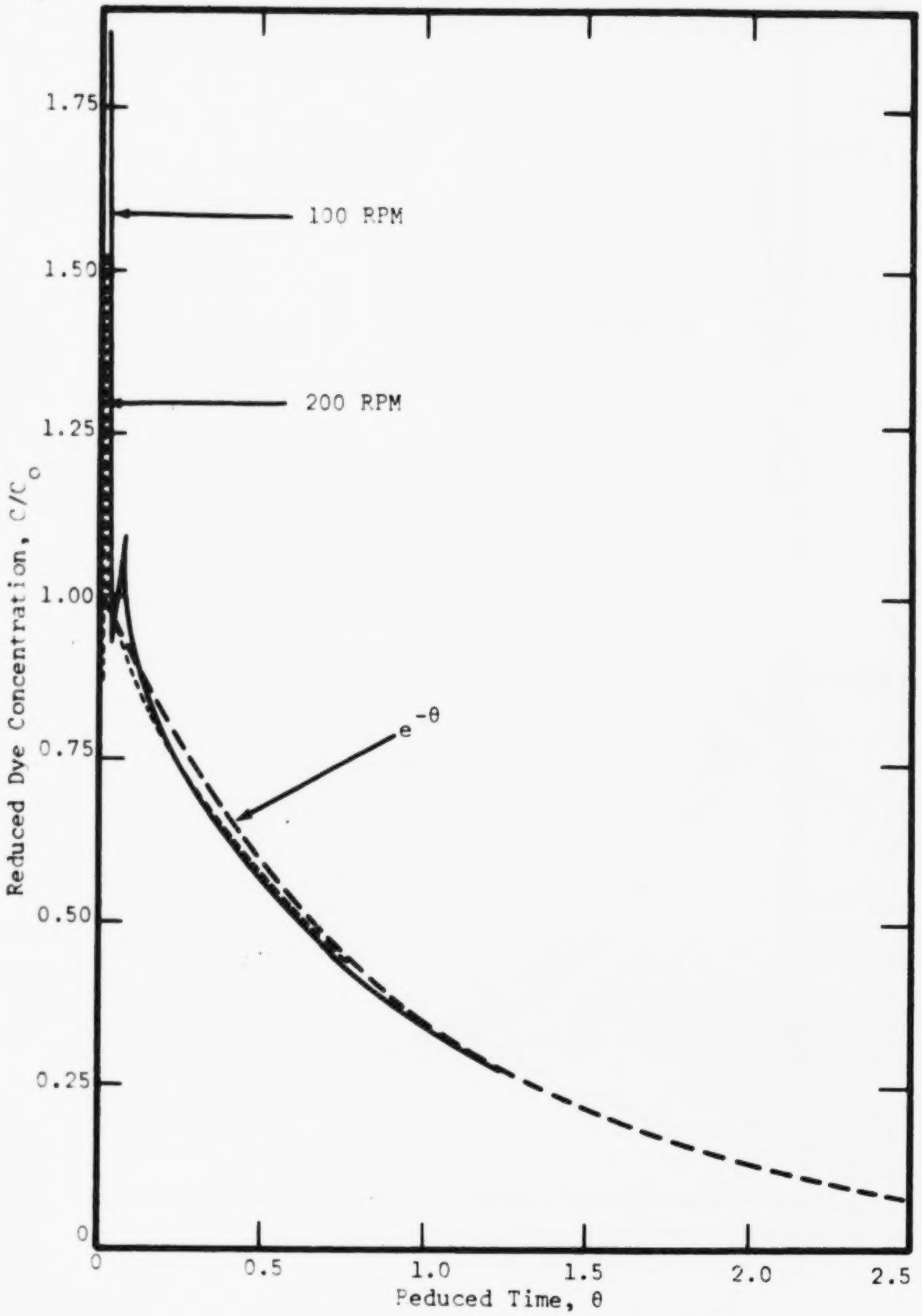

FIGURE 17. RESIDENCE TIME DISTRIBUTION CURVES FOR MIXING SPEEDS OF 100 AND 200 RPM

as a measure of the deviation from ideal mixing, the results of mixing speeds may be assessed. Since the high initial peaks shown in Figures 16 and 17 are a result of short-circuiting, this approach appears to provide a reasonable means for comparison. From a graphical integration of the area produced from short circuiting it was found that deviation from ideal mixing decreased from 9 percent at 25 rpm to approximately 0.5 percent at 200 rpm. These data, along with the percent deviation at 50 and 100 rpm are shown in the log-log plot of mixing speed vs. the percent of the distribution curve above $e^{-\theta}$ in Figure 18. No implication is intended from the log-log relation; it is simply used as a convenient means of plotting the data. The results of the residence time distribution investigation may be summarized as follows:

1. Essentially complete mixing was achieved at 50 rpm (3 percent deviation).

2. Improvements in mixing characteristics diminished as the mixing speed was increased. (6 percent improvement from 25-50 rpm vs. 0.1 percent improvement from 175-200 rpm.)

FILM ACCUMULATION

Procedure

The effect of mixing speed on biological film accumulation was studied by developing films at 50, 100, 150 and 200 rpm. During these studies three reactors were innoculated with 400 ml of settled sewage from the Clemson University Treatment Plant. The remainder of the one liter reactor was then filled with 100 ml of concentrated glucose feed (7000 mg/l), 50 ml of 1 M phosphate buffer (pH = 7.0) and 450 ml of tap

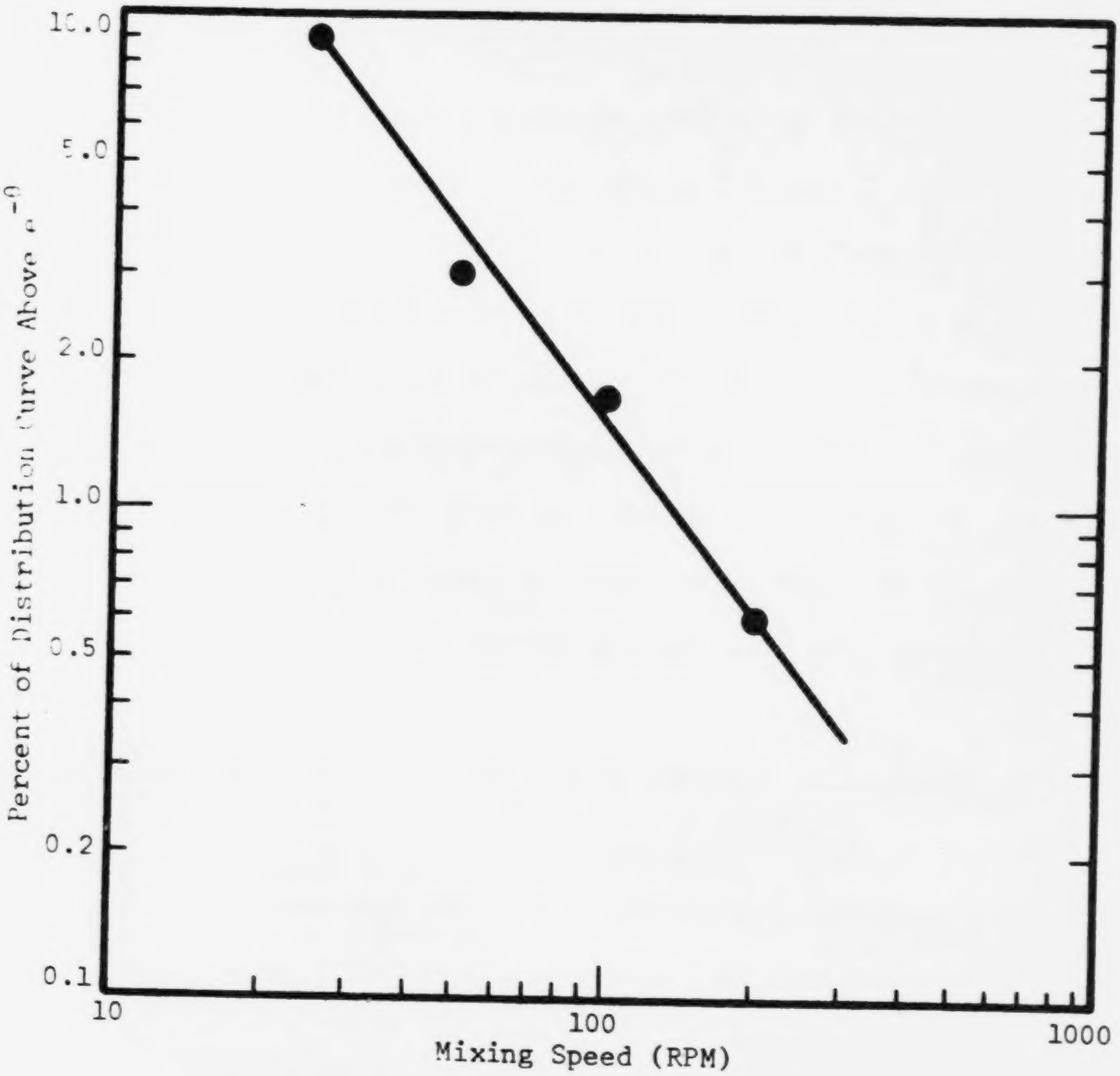

FIGURE 18. SHORT CIRCUITING AS A FUNCTION OF MIXING SPEED

water. The reactors were then operated as a batch system for approximately 24 hours to allow wall growth to develop. Aerobic conditions were maintained during this period by an air stream which entered through the influent substrate lines and was dispersed through the liquid by constant mixing. At the end of the 24-hour period the air flow was stopped and continuous flow initiated at 15 to 20 ml/min. During the following 24-hour period the substrate flow was increased in four increments to 134 ± 3 ml/min. At this flow rate the retention time of 7.5 min. was low enough to insure that all suspended organisms were washed out and only the attached organisms remained.

After obtaining the desired flow rate the influent substrate concentration was adjusted to 215 ± 20 mg/l of glucose and the mixing motor adjusted to one of the test speeds (50, 100, 150 or 200 rpm).

The effects of mixing speed on biological film accumulation were assessed from measurements of total film thickness, substrate utilization and suspended solids. These measurements were initiated when the desired flow rate and substrate concentration were achieved (time = 0) and continued until sloughing occurred. By determining the biological film accumulation and resulting substrate removal over a period of time for various mixing speeds two pertinent facts could be evaluated. First, the active film thickness could be determined as a function of mixing speed. Second, the plateau film thickness could be evaluated. The plateau film thickness is that thickness at which hydraulic shear prevents further accumulation. The plateau thickness should, therefore, vary with mixing speed and was of primary interest in this investigation.

Results and Discussion

From studies conducted at 50, 100, 150 and 200 rpm, it was found that the accumulation of biological films on surfaces was in general agreement with the results reported by Sanders (56). At each test speed the biological films increased from essentially zero thickness until a plateau thickness was reached. This plateau thickness was maintained until sloughing occurred. It was found that the plateau film thickness and the uniformity of the biological films were independent of mixing speed but the critical film thickness was not.

As shown in Figure 19, the development of biological films encompassed three distinct periods of growth. These data represent the average film thickness, substrate removal and volatile suspended solids observed as a function of time for a mixing speed of 100 rpm. Each point represents the average results for three reactors except for a few samples where premature sloughing occurred. The first period of growth was logarithmic and included the period of growth from zero thickness until the "critical" or "active" film thickness was attained. At the active thickness, substrate utilization reached a steady-state value, and was not affected by additional film accumulation. The logarithmic phase was followed by a period of near constant growth which terminated when the shear on the biological film prevented further accumulation. The last period was the plateau phase in which new cell growth equaled the biological film being washed from the walls by the shear of the adjoining liquid, and the film thickness remained constant. There are four pertinent facts which should be noted from Figure 19. First, the substrate removal reached a steady-state value after a film thickness of approximately 70 microns was reached although

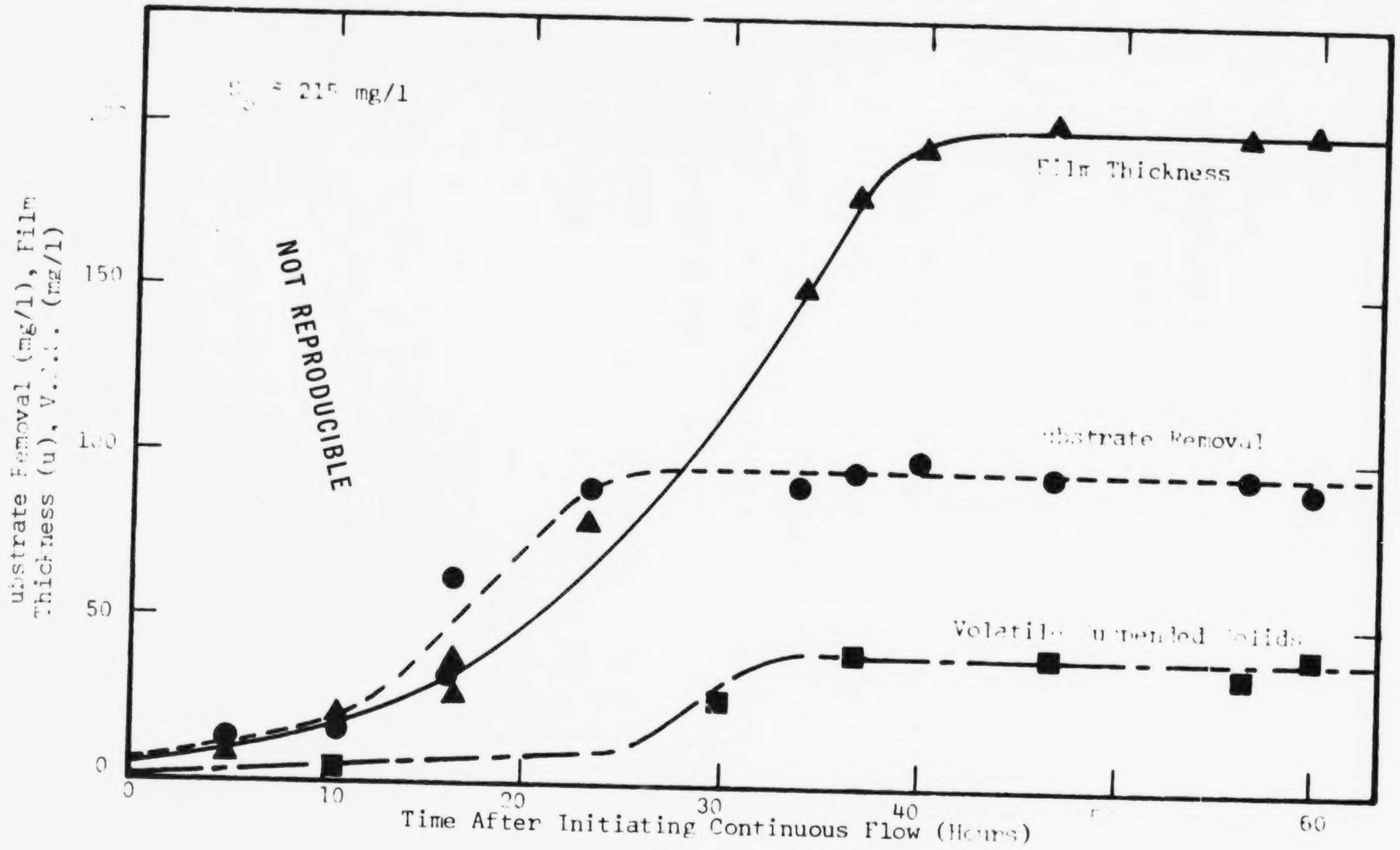

FIGURE 19. BIOLOGICAL FILM ACCUMULATION, SUBSTRATE UTILIZATION AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 100 RPM

the film thickness continued to increase. Second, a complete film layer was developed prior to the attainment of steady-state with respect to substrate utilization. Third, suspended solids in the effluent were negligible until the plateau thickness was approached, and fourth, there was no detectable increase in substrate utilization with an increase in suspended solids.

The first two points may be more easily grasped from a plot of total film thickness vs. substrate removal shown in Figure 20. A plot similar to Figure 20 was used to determine the "active thickness," which is numerically equal to the thickness where utilization becomes independent of the total film accumulation. These data represent the results obtained at 50 and 200 rpm and illustrate two important points. First, the steady state substrate utilization increased with mixing speed and second, the maximum film accumulation decreased with mixing speed. The increase in substrate utilization is readily apparent. The removal increased from 75 mg/l at 50 rpm to 103 mg/l at 200 rpm. The effect of mixing speed on film accumulation is somewhat more subtle. At 200 rpm, film accumulations above 100 μ in thickness were prevented by hydraulic shear. After reaching 100 μ, the film thickness remained constant and was very uniform. At a mixing speed of 50 rpm greater film accumulations were observed and the film surface was more uneven. Film thicknesses as high as 484 μ were observed with an average thickness of 300 μ.

The data for 100 and 150 rpm were plotted in a similar manner and the critical thickness determined for each test speed. These results and the plateau film thickness at the various mixing speeds are represented in Figure 21. It should be noted that the active film

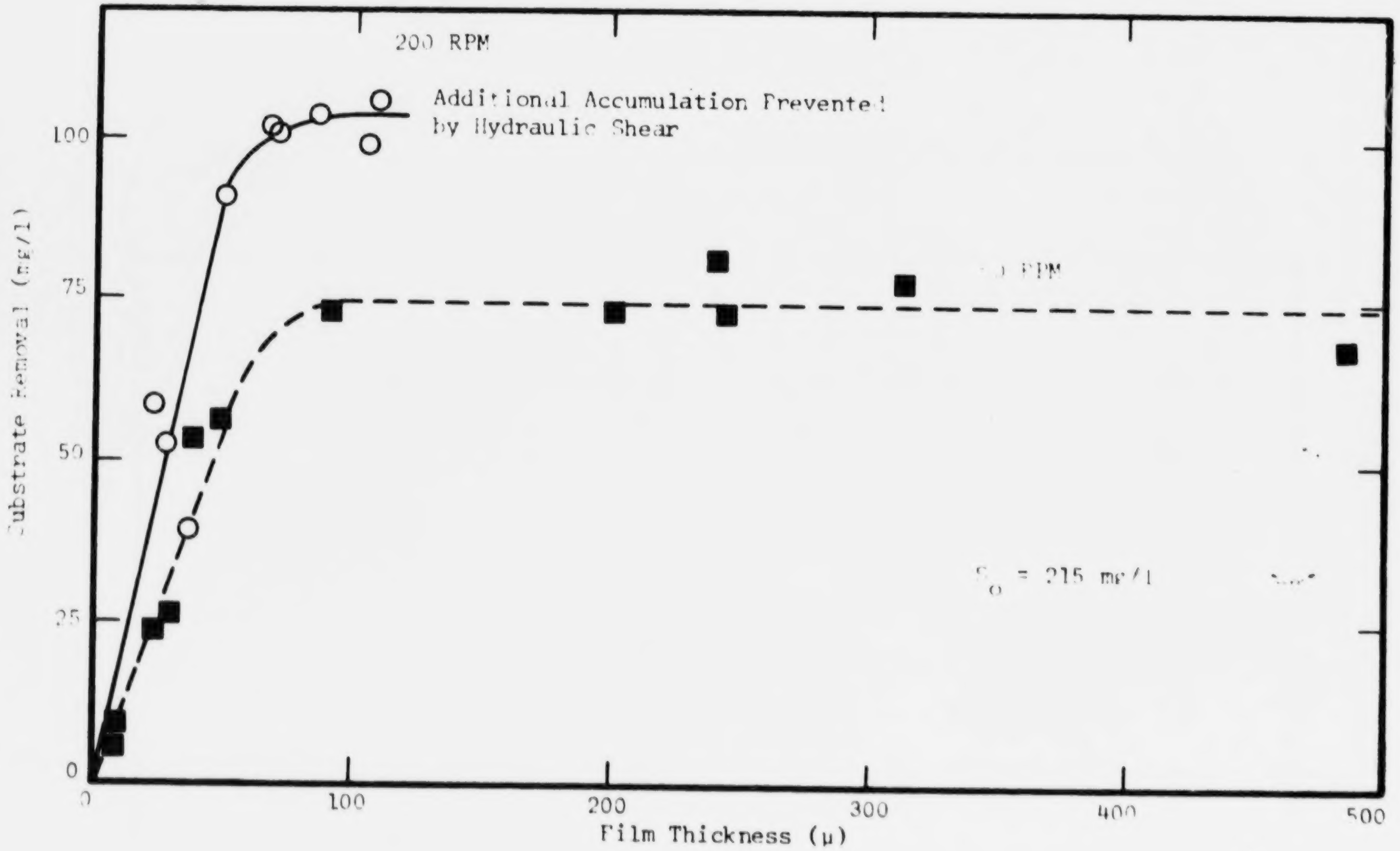

FIGURE 20. DETERMINATION OF THE ACTIVE FILM THICKNESS AT 50 AND 200 RPM

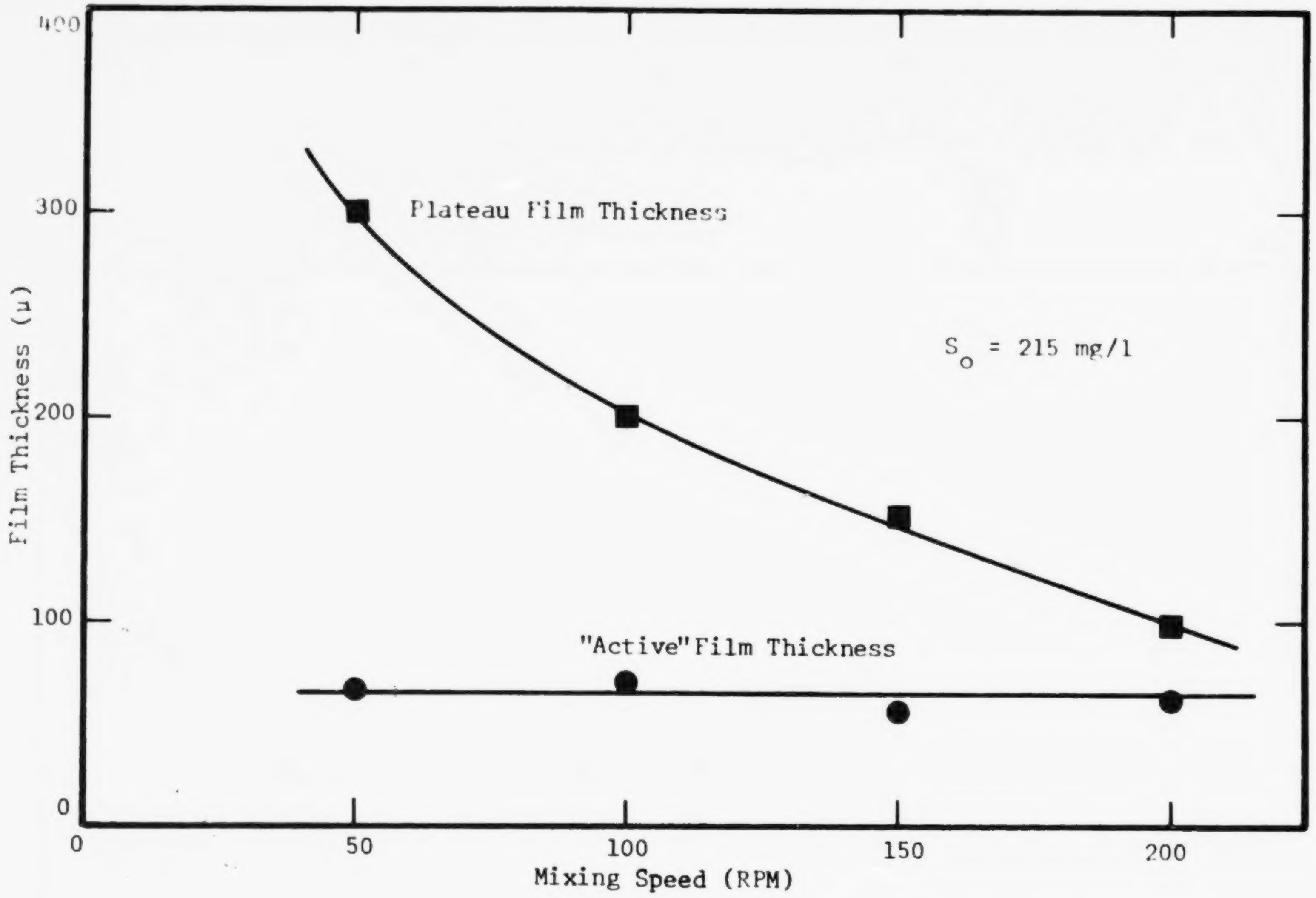

FIGURE 21. THE EFFECT OF MIXING ON THE ACTIVE AND PLATEAU FILM THICKNESS

thickness was essentially independent of mixing speed while the plateau thickness decreased with increased mixing, and actually approached the active thickness at mixing speeds above 200 rpm. The active thickness varied from a high of 73 μ to a low of 55 μ. The weighted active film thickness was approximately 70 μ as reported for the 100 rpm mixing study. The most important point, however, was the fact that no significant correlation was found between mixing speed and active film thickness at a 95 percent confidence limit.

At mixing speeds above 50 rpm the film was uniform and clearly defined, but at 50 rpm considerable variation was observed. Individual steady-state film measurements were observed at 50 rpm which varied from 202 to 484 μ so the plateau thickness of 300 μ represents an average value. The plateau thickness for the other mixing speed was determined from plots similar to Figure 19 and was 200, 152, and 97 μ for mixing speeds of 100, 150 and 200 rpm, respectively. One reason for the problems encountered in determining the plateau film thickness at 50 rpm was the nature of the film surface. As the mixing speed was decreased the films became more uneven as well as thicker. The difference in film texture at various mixing speeds was apparent from microscopic examination.

LIQUID FILM DIFFUSION

Procedure

The importance of liquid film diffusion was studied in both steady-state and transient operations. During both series of tests the influent substrate concentration was maintained at 215 ± 20 mg/l.

During the steady-state operations, films were developed at 50, 100, 150 and 200 rpm until the plateau thickness was attained and then substrate utilization and biological growth were measured. Since the only variable was the mixing speed any change in substrate removal should indicate a resistance to diffusion by a stagnant liquid film.

Since there was a possibility that a difference in biological film characteristics, rather than liquid film diffusion, would produce differences in utilization at various speeds, a transient study was also made. In this study biological films were developed in three reactors at 225 rpm. After steady-state was attained the mixing speed of all reactors was reduced at random to one of the test speeds (25, 50, 75, 100, 125, 150, 175 or 200 rpm). Thirty minutes after reducing the mixing speed the oxygen concentration and substrate removal were determined. The mixing speed was then returned to 225 rpm for a 15-minute period to eliminate any cells which may have accumulated during the 30-minute test period. This procedure was repeated until each of the test speeds had been tested. Since the test speeds were selected at random to eliminate time as a variable, some mixing speeds were tested more than once.

Results and Discussion

The observed results of both the steady-state and transient studies shown in Figure 22 clearly indicate that substrate removal increased with mixing speed. The relation was not linear and the steady-state results indicate that little improvement in substrate removal was obtained at mixing speed, above 100 rpm. When the mixing speed was increased from 50 to 100 rpm the substrate removal increased from 75 mg/1 to 96 mg/1, or 28 percent. However, an increase in mixing

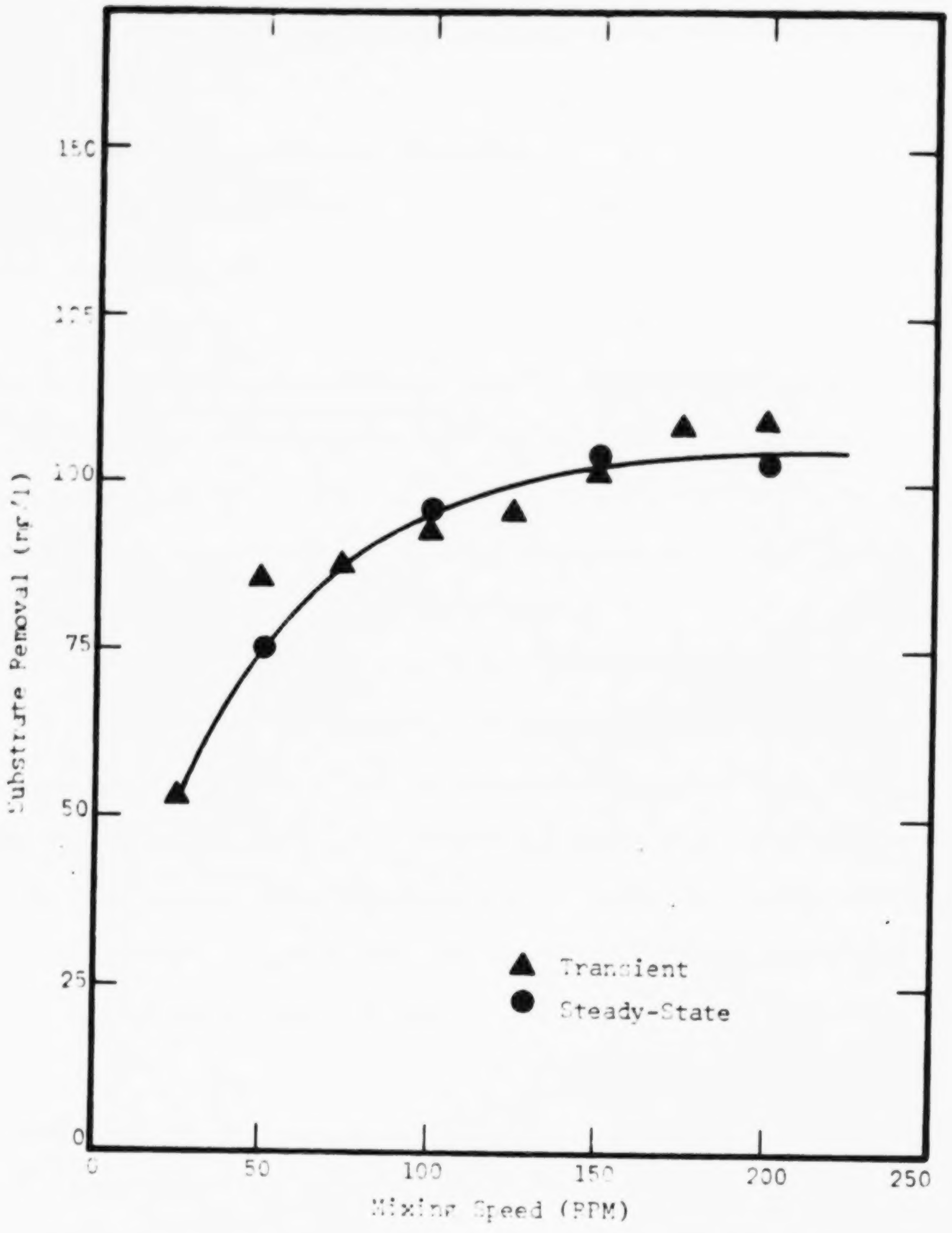

FIGURE 22. THE EFFECT OF MIXING ON SUBSTRATE REMOVAL

speed from 100 to 200 rpm resulted in an increased substrate removal of only 7.3 percent.

Although the transient studies followed the same trend as the steady-state results, three additional benefits were provided. First, the possibility that differences in substrate removal at various speeds was due to changes in biological film characteristics rather than liquid film diffusion was dispelled. Any changes in biological film which could occur in the 30-minute test period should be negligible. Second, time was removed as a possible variable by testing in a random fashion and third, the agreement between the "transient" and steady-state results indicates that fixed-film reactors respond very rapidly. This point is demonstrated more fully in a later section.

Since the influent substrate concentration was held constant during both studies the concentration in the reactors decreased as the rate of substrate utilization increased. This test procedure may have masked the effect of mixing to some extent, but it remained obvious that liquid velocity is a factor which must be considered in fixed film reactors. This same conclusion was also reached by Swilley et al. (106), and Maier et al. (107).

The observed increase in substrate utilization was apparently due to a decrease in resistance to diffusion in the liquid film. This decreased resistance produced a higher substrate concentration in the biological mass and increased the overall rate of the process. However, it would appear that a simple liquid film diffusion model would be an oversimplification of the process. It was found in this and other studies (55, 56) that the biological process does not involve a simple surface reaction. To the contrary, a finite thickness of biological film is involved which offers a resistance to diffusion in addition to that of the liquid film. Furthermore, it will be seen in another section of this report that the growth rates for fixed film reactors are of the same order of magnitude as those reported for dispersed cells by Pirt (108) and McWhorter and Heukelekiam (109) for glucose limited systems. This indicates that the biological reaction is not so rapid that it may be considered instantaneous and neglected in the overall process. It therefore appears that the removal of substrate by biological films involves several reactions in series, and may be expressed in an overall reaction which includes both diffusion and biological growth. Actually, the Monod saturation function accomplishes this goal if K_g is taken as a velocity dependent variable.

Since the term K_g is defined as that bulk concentration required to produce a growth rate equal to one-half the maximum, one would expect this value to change with diffusional resistance. A similar conclusion was reached by Lilly and Sharp (110) for enzymes attached to polymers. In these studies the activity of chymotrypsin attached to carboxy-methylcellulose particles was studied in a completely mixed batch reactor. The purpose of this investigation was to determine the effect of mixing speed on the Michaelis-Menten constant K_m. It was found that the value of the observed "constant" varied with both mixing speed and substrate concentration. This was called the apparent constant K_m' and was related to the true constant by the equation

$$K_m' = K_m + \delta V_m'/D_L \qquad (68)$$

where K_m' = apparent Michaelis-Menten constant (M/L^3)

K_m = Michaelis-Menten constant (M/L^3)

δ = thickness of diffusional boundary layer (L)

V_m' = apparent maximum velocity of reaction (M/L^3)

D_L = diffusivity of substrate in reaction solution (L^2/T).

Marlar (111) also reported that the mixing speed had an effect on the saturation constant. In studying the effect of turbulence on bacterial substrate utilization Marlar found that the Monod (3) saturation constant K_g could be expressed as

$$K_g = (7.84)\ 10^{-0.01115 k_L a} \tag{69}$$

where K_g = apparent saturation constant (M/L^3)

$k_L a$ = oxygen transfer coefficient (T^{-1}).

It should be noted that the value of 7.84 mg/l does not represent the true saturation constant. The saturation constant of 7.84 mg/l would only occur at a $k_L a$ value of zero and, therefore, represents the maximum apparent saturation constant. However, Equation 72 does show that the saturation constant K_g decreases with mixing speed since the transfer coefficient $k_L a$ increases with mixing. The maximum specific growth rate constant μ_{max} was reported to be constant throughout the investigation.

If for the moment one assumes that the equation

$$F\ (S_0 - S_1) = \frac{\mu_{max}}{Y}\ (d)(A)(X) \left[\frac{S}{K_g + S} \right] \tag{61}$$

NOT REPRODUCIBLE

is valid and the maximum specific growth rate is a constant, the relative values of K_g may be calculated for mixing speeds of 50, 100, 150 and 200 rpm. For this calculation the saturation constant at a mixing speed of 100 rpm, K_{g100} was assumed to be 100 mg/l. Based on this assumed value and the observed results of the steady state diffusion study previously reported, it was found that the ratios of K_g/K_{g100} were approximately 1.05, 0.75 and 0.65 at mixing speeds of 50, 150 and 200 rpm, respectively. Additional values of K_{g100} were assumed to see if the ratio K_g/K_{g100} varied for different values of K_{g100}. It was found that this ratio was dependent on the initial value of K_{g100} as shown in Figure 23. Figure 23 graphically demonstrates both the importance of liquid film diffusion and a means of incorporating this variable into a mathematical model. The shape of the curves is particularly interesting. If K_g is a measure of the total resistance of the process, that portion of the resistance attributable to liquid film diffusion may be evaluated. At mixing speeds above 200 rpm the value of K_g ceases to diminish with an increasing velocity as indicated by the near horizontal shape of the curve. This indicates that liquid film diffusion has been minimized and the remaining resistance must be primarily due to the biological reaction and diffusion into the biological film. This point will also be discussed in a later section, but at this time the more important goal was to establish a constant mixing speed.

DETERMINATION OF THE MIXING SPEED

The results of the mixing studies provided a means of determining the mixing speed for the remainder of the investigation. A

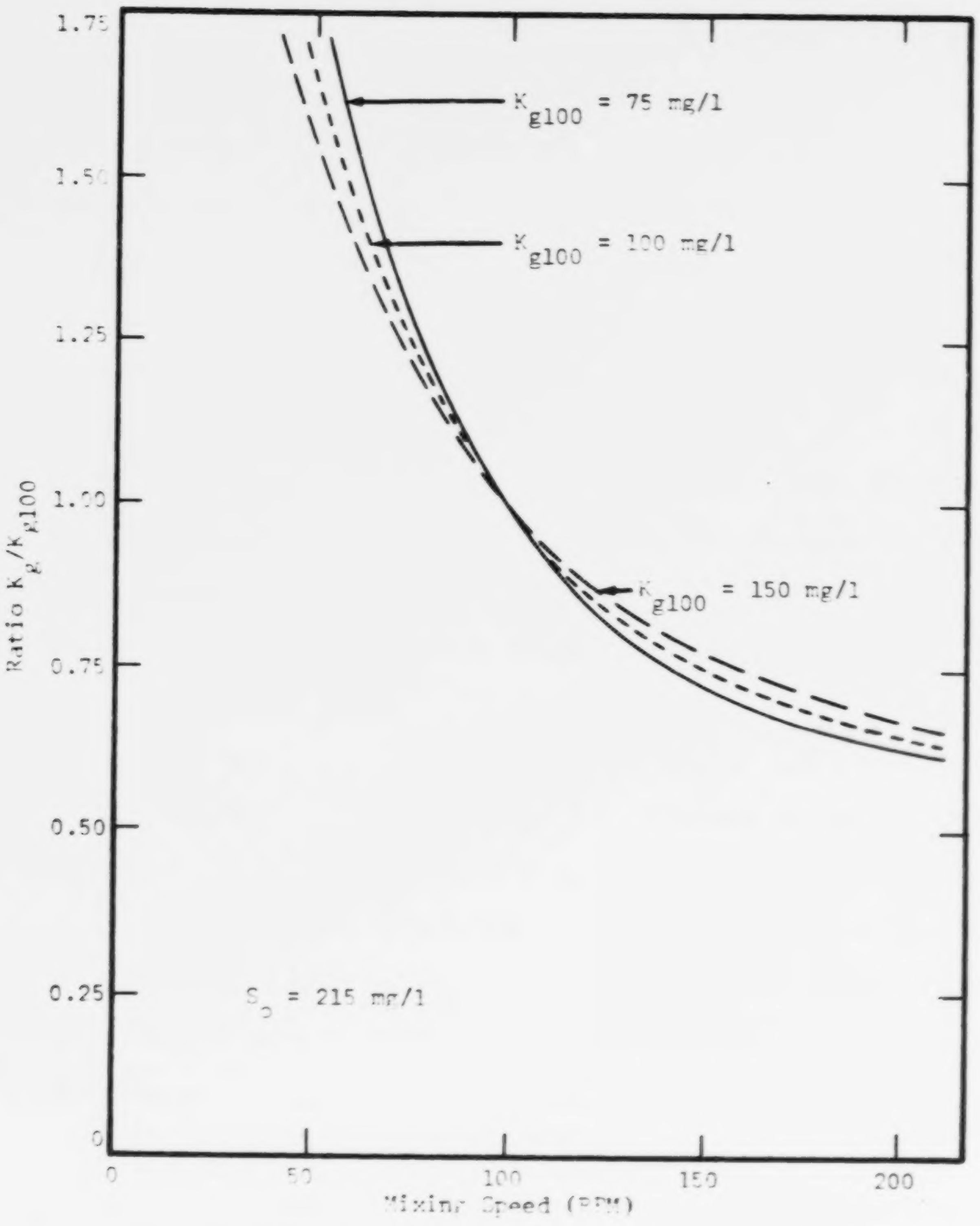

FIGURE 23. THE EFFECT OF MIXING SPEED ON THE SATURATION CONSTANT K_g

speed of 25 rpm did not sufficiently approximate complete mixing and the 3 percent deviation at 50 rpm was more than desired. In addition, the film surface at 50 rpm was very uneven. At 100 rpm a smooth film surface was produced, substrate utilization rates were in a convenient range and mixing was essentially complete. At mixing speeds greater than 100 rpm there was little improvement in either mixing characteristics or substrate removal. Furthermore, at mixing speeds above 100 rpm the plateau and film thickness were so close that problems could arise in defining all stages of biological film accumulation. Therefore, the mixing speed selected was 100 rpm.

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VI. SINGLE STAGE, COMPLETELY MIXED, FIXED FILM REACTORS

GENERAL

Before attempting to model plug-flow, fixed film reactors, several single stage kinetic studies were conducted. The objective of these studies was to delineate the role of substrate and dissolved oxygen concentrations on the degradation of organics and the production of biological mass. The specific points of interest were:

(1) The effect of the dissolved oxygen concentration on the active film thickness.

(2) The effect of dissolved oxygen on the rate of substrate removal.

(3) The effect of substrate concentration on the rate of substrate removal.

(4) The feasibility of describing fixed film reactors with the continuous culture theory.

In view of several reports (35, 36, 56) which attribute dissolved oxygen with controlling the thickness of the active microbial layer, it was decided that this variable should be investigated. It was clearly recognized that under certain conditions dissolved oxygen may be the nutrient which limits the rate of biological growth, but this was not the region of interest. The problem here was to ascertain if oxygen can limit the thickness of the active microbial mass when substrate is the nutrient controlling biological growth. This

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assertion has often been made in connection with biological filters but seems to lack a firm scientific basis.

Substrate was obviously included because the rate of biological growth and waste degradation is usually a function of substrate concentration. Due to the dilute nature of most waste streams, substrate concentrations are normally below that required for maximum growth. In addition, this study was designed to verify or disprove mathematical models for the prediction of substrate removal in completely mixed, fixed-film reactors.

During this entire kinetics study three reactors were operated in parallel and under the same conditions for each test as outlined in Chapter IV. The pH was 6.5 ± 0.1, the temperature 25 ± .5°C, and the flow rate 134 ± 3 ml/min. During the first portion of this study, the substrate was held constant for various dissolved oxygen values and in the second portion, substrate was the variable of interest.

DISSOLVED OXYGEN CONCENTRATION

Procedure

In studying the effect of the dissolved oxygen concentration on fixed-film biological reactors, two series of tests were conducted. In the first series of tests, the development of the biological film was studied at residual dissolved oxygen concentrations of 2.5, 6, 8 and 15 mg/l and the active film thickness determined. For these tests three reactors were innoculated with a mixed population of microorganisms and operated as a batch system until wall growth developed. Continuous flow was then instituted over a 24-hour period until a flow of 134 ± 3 mg/l was attained at an influent substrate concentration of

215 ± 20 mg/l. The dissolved oxygen concentration was then adjusted to and maintained at 2.5 mg/l by regulating the oxygen content of the gas mixture flowing into the dilution water tank. The development of the biological film was followed from essentially zero film thickness until sloughing occurred. The substrate utilization was determined as the film thickness increased in order to determine the active thickness as described in Chapter V. The test was then repeated at dissolved oxygen concentrations of 6, 8 and 15 mg/l. This provided a means of determining the active film thickness as function of the dissolved oxygen concentration.

In the second series of tests, the biological films were developed at a D.O. concentration of approximately 2.5 mg/l as described above until steady state was attained. The dissolved oxygen concentration was then increased in increments up to 18 mg/l and the substrate utilization determined at each level. The reactors were operated for at least one hour at each dissolved oxygen concentration before sampling. If the dissolved oxygen concentration influenced either the active film thickness or microbial growth, then an increase in the dissolved oxygen concentration should produce a corresponding increase in substrate utilization.

Results and Discussion

Both studies designed to delineate the role of dissolved oxygen in fixed film biological reactors showed that the dissolved oxygen concentration had no effect on the active biological film thickness or rate of substrate removal. This first point is clearly demonstrated in Figure 24 where the active film thickness is plotted as a function of the residual dissolved oxygen concentration.

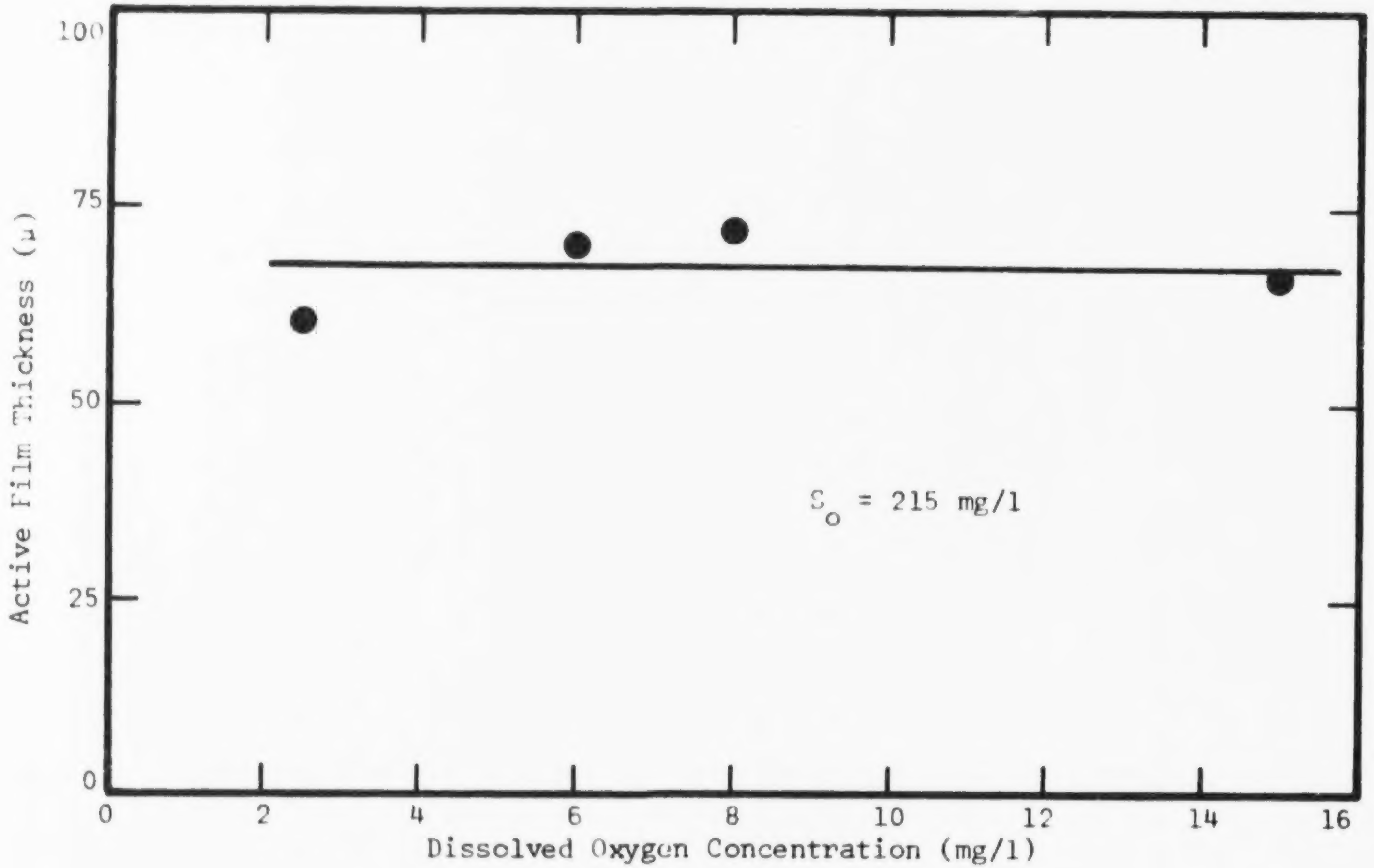

FIGURE 24. THE ACTIVE FILM THICKNESS AS A FUNCTION OF DISSOLVED OXYGEN

There was some variation in active thickness, but this was not in agreement with Equation 36. This would require that the film thickness at 6, 8 and 15 mg/l be 1.6, 1.8 and 2.5 times that at 2.5 mg/l, respectively. This condition was not met. In fact, at a 95 percent confidence limit there was no significant correlation between active film thickness and dissolved oxygen concentration. This shows that the active thickness and aerobic thickness reported by Schulze (35) are not necessarily one and the same. This conclusion was supported by microscopic examinations of the biological film. When the active film thickness was reached, no dark zone characteristic of anaerobic conditions were observed. At mixing speeds above 100 rpm these dark zones seldom appeared. They were only apparent when lower mixing speeds were used and the film accumulation exceeded several hundred microns in thickness.

The rate of substrate removal was also independent of the dissolved oxygen concentration. This is shown in Figure 25 where the rate of substrate utilization is plotted as a function of dissolved oxygen concentration. Although the dissolved oxygen concentration was increased from 2.5 to 18 mg/l the deviation in substrate utilization was only 4.5 percent. This is within the range of experimental error. If the dissolved oxygen concentration had influenced either microbial growth or the active film thickness, a significant change in substrate utilization should have resulted. No change occurred. Figure 25 clearly demonstrates that the dissolved oxygen concentration did not affect substrate utilization in the region of interest.

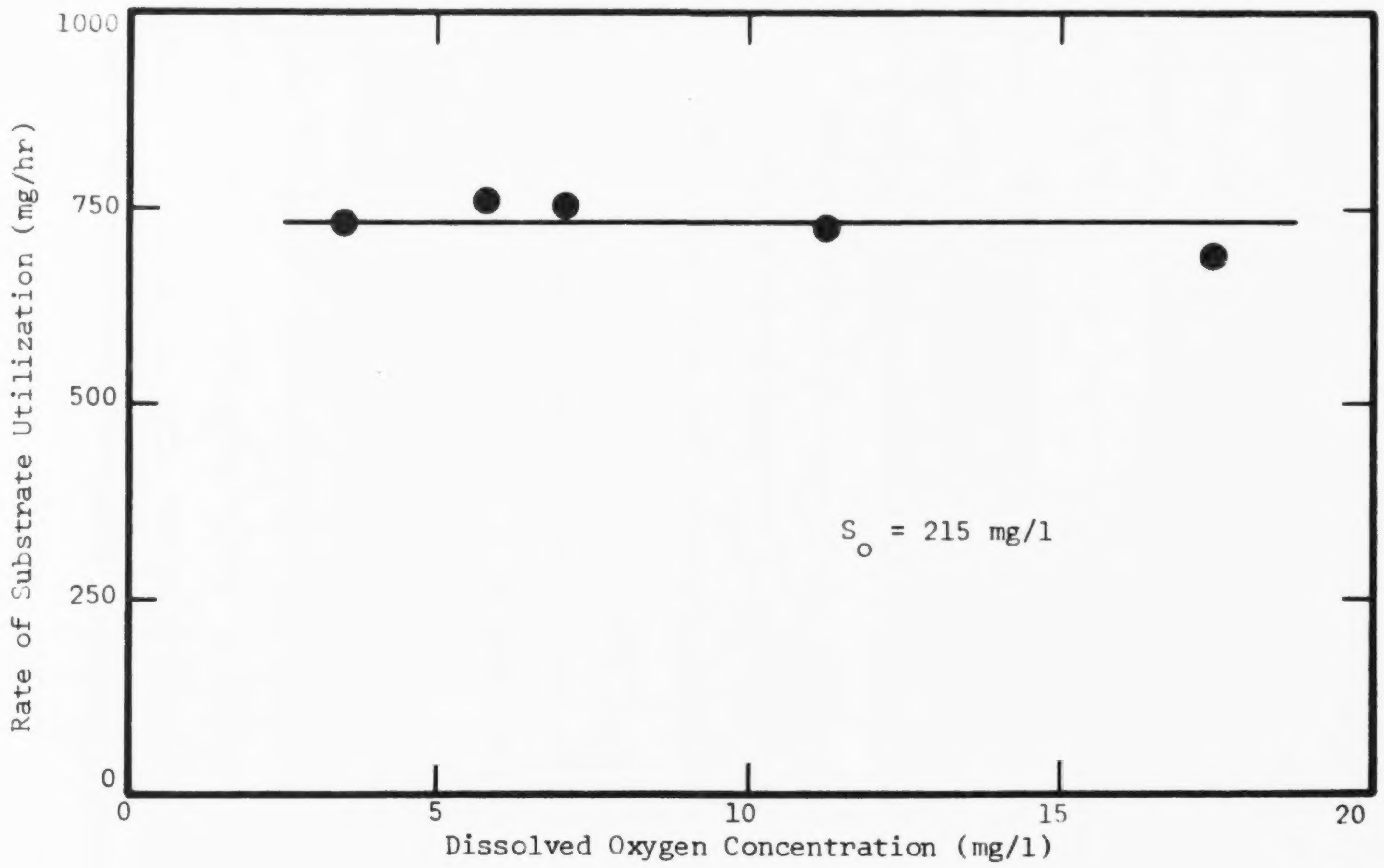

FIGURE 25. THE EFFECT OF DISSOLVED OXYGEN ON SUBSTRATE UTILIZATION

These results should not be confused with studies conducted under conditions in which dissolved oxygen was the controlling nutrient such as those performed by Mueller et al. (69). Mueller, Boyle and Lightfoot (69) and Rahn and Richardson (67) worked with excess concentrations of carbon substrate and dissolved oxygen was the nutrient controlling biological growth. Therefore, their results cannot be compared to this study. However, these results are at variance with reports by Schulze (35) and Eckenfelder (36) which describe filter performance in terms of the carbon substrate and contend that oxygen controls the active microbial film. This assertion implies that carbon substrate controls the rate of biological growth and yet oxygen limits the thickness of the active film. In view of the relative rates of substrate and oxygen diffusion, and the rates of utilization in the biological film, the existence of such a condition is doubtful and was not found in this study. To the contrary, it was found that oxygen did not influence the process at all under conditions in which organic substrate controlled the rate of microbial growth.

Although it is realized that any nutrient may control the rate of biological growth, the determination of the relative substrate and dissolved oxygen concentration at which oxygen becomes growth limiting was beyond the scope of this study. However, it should be pointed out that in several of the tests conducted in the preliminary studies dissolved oxygen values below 0.5 mg/l were attained with no adverse effect on substrate removal or organism production.

SUBSTRATE CONCENTRATION

Procedure

The effect of substrate concentration on biological growth and substrate removal was studied at steady-state substrate concentrations from 27 to 440 mg/l. For these tests three annular reactors were innoculated with a mixed population of microorganisms from settled sewage and then brought to steady-state conditions at a residual glucose concentration of approximately 135 mg/l. The concentration was then changed to one of the test concentrations and allowed to operate for a period of approximately 6 hours before sampling, and only then if steady-state were attained as indicated by a constant oxygen utilization. The glucose utilization and organism production were then determined. Since the film thickness remained constant the organism production was determined by measuring the mass of organisms being washed from the walls. During these tests the dissolved oxygen concentration was maintained between 6 and 9 mg/l. Since the dissolved oxygen concentration had no effect on either substrate removal or active film thickness, a more stringent control was not required.

Results and Discussion

When the influent substrate concentration was increased both the rate of substrate removal and biological growth increased as expected. The rate of substrate removal did not follow an elementary first or second order reaction, but obeyed a saturation function as predicted from the equation

$$F\,(S_0 - S_1) = \frac{\mu_{max}}{Y}\,(A)(X)(d)\left[\frac{S_1}{K_g + S_1}\right] \qquad (61)$$

All of the values in Equation 61 were determined from actual measurements except the maximum specific growth rate μ_{max} and saturation constant K_g. The flow, F was maintained at 134 ± 3 ml/min and both the influent and effluent substrate concentrations were determined in triplicate. The total exposed surface area of 2000 sq. cm. was available for biological growth so the value of A was known. Furthermore, the average biological film thickness at 100 rpm was found to be 70 μ as reported in Chapter V.

The unit mass of biological film was found by determining the dry weight of film on a removable slide. Since the initial film thickness and slide area were known the unit mass, on a dry weight basis could be calculated. To accomplish this the wet film thickness of a removable slide was carefully measured and the slide then placed in a dessicator for 48 hours. At the end of this period the slides were weighed to determine the dry weight of film and removable slide. Table 3 gives the observed results for 14 determinations. The average unit mass of biological film on a dry weight basis was 95 mg/cc with a standard deviation of 18.8 mg/l.

The yield, Y was determined by dividing the volatile solids produced by the glucose consumed. As shown in Table 4 this was found to be 0.26 gms of cells per gram of glucose consumed. The yield was determined after a steady-state film thickness was attained so the cells being formed were washed from the walls by hydraulic shear. Under these conditions the suspended solids and organism growth were equal. It should also be noted from Table 4 that the maximum quantity of suspended solids in the reactor during this test was 40.2 mg.

TABLE 3

DETERMINATION OF THE UNIT MASS OF BIOLOGICAL FILM FROM DRY SLIDE WEIGHTS

	Dry Weight of Film on Slide (mg)	Percent of Slide Covered (%)	Wet Film Thickness ( )	Unit Mass of Film (mg/l)
	124.0	100	180	119.0
	108.3	100	200	93.0
	82.4	100	190	74.7
	123.0	100	160	130.0
	93.1	80*	190	105.0
	81.5	80*	210	83.4
	104.0	100	200	89.4
	68.0	80*	170	87.2
	82.4	90*	160	98.7
	76.0	100	200	65.5
	102.0	80*	190	115.5
	83.0	100	180	79.4
	92.0	100	190	83.4
	126.0	100	200	109.0
Total	1445.7	1310	2620	1333.3
Average	103	94	187	95

Standard Deviation = 18.8 mg/l
Area of Slide = 9 sq. inches = 58 cm^2.

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[*] When the removal of the slide from the reactor produced irregular edges one or both edges were trimmed to produce a known coverage.

TABLE 4

DETERMINATION OF THE AVERAGE YIELD FOR RUN NUMBER 9

Substrate Removal (S_0-S_1) mg/l	Cell Production		Percent Volatile	
	Total (T.S.S.) mg/l	Volatile (V.S.S.) mg/l	$100 \frac{\text{V.S.S.}}{\text{T.S.S.}}$ %	Yield V.S.S./(S_0-S_1)
132.6	34.8	32.0	92.1	0.241
120.0	40.2	35.6	88.7	0.296
46.3	12.9	12.1	94.2	0.262
122.6	35.9	32.9	91.6	0.269
108.0	26.8	23.6	87.9	0.219
55.0	11.5	11.2	96.2	0.205
108.8	26.6	25.0	94.3	0.230
119.2	28.5	25.0	87.6	0.210
48.5	16.9	15.5	92.0	0.319
89.6	30.2	28.8	94.7	0.322
		Average	91.9	0.257

Compared to the 1330 mg of film attached to the 2000 sq cm of surface area and extending through the active thickness of 70 μ the suspended solids were only 3 percent of the active mass. Suspended solids were, therefore, negligible in terms of glucose consumption.

In order to determine the saturation constant K_g and the maximum specific growth rate constant μ_{max} the steady-state removal equation

$$F\ (S_0-S_1) = \frac{\mu_{max}}{Y}\ (A)(X)(d)\left[\frac{S_1}{K_g + S_1}\right] \qquad (61)$$

was rearranged to provide the linear form

$$\frac{(A)(X)(d)}{FY\ (S_0-S_1)} = \frac{1}{S_1}\ \frac{K_g}{\mu_{max}} + \frac{1}{\mu_{max}} \qquad (70)$$

When the left side of Equation 70, which is equal to the reciprocal of the specific growth rate, was plotted against the reciprocal of the substrate concentration, a linear plot resulted as shown in Figure 26. The slope of the line is equal to K_g/μ_{max} and the ordinate intercept is $1/\mu_{max}$. From a regression analysis of these data K_g was found to be 121 mg/l and μ_{max} was 0.28 hr^{-1}.

When these values for μ_{max} and K_g were substituted into the substrate removal equation with the measured values of the other parameters the theoretical substrate removal curve shown in Figure 27 was produced. The theoretical curve predicts the rate of substrate removal to be expected at various substrate concentrations for the conditions used in this study. Also shown in Figure 27 are the experimental points obtained when the reactors were operated at steady-state substrate concentrations from 27 mg/l to 440 mg/l of glucose. Three points should be noted. First, the rate of substrate removal approaches a constant value as the substrate concentration is increased. A saturation phenomenon is definitely exhibited. Second, the effluent concentration is not independent of the influent concentration as is the case for completely mixed, slurry reactor systems.

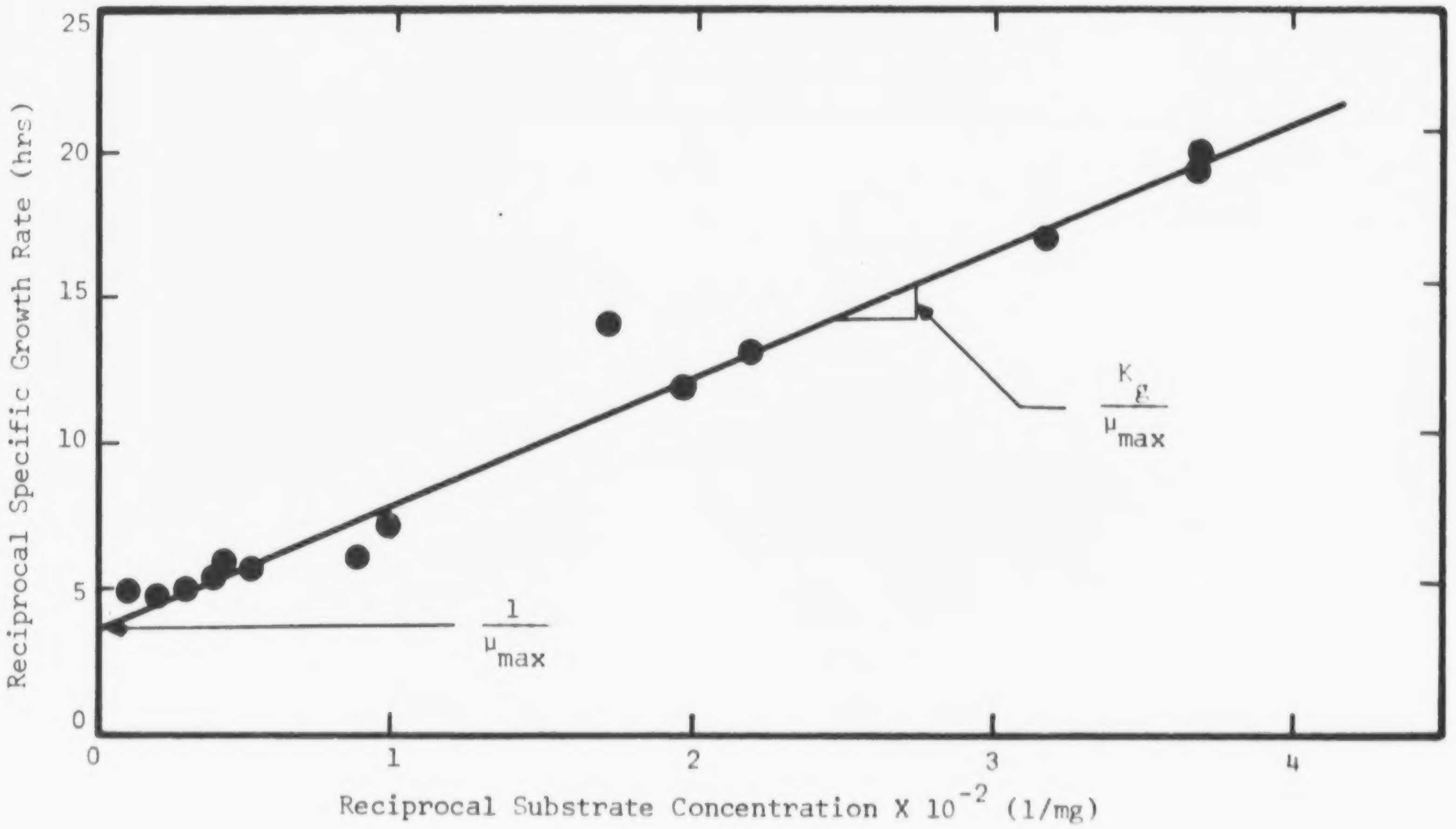

FIGURE 26. DETERMINATION OF THE BIOLOGICAL CONSTANTS K_g AND μ_{MAX} FOR SINGLE STAGE REACTORS

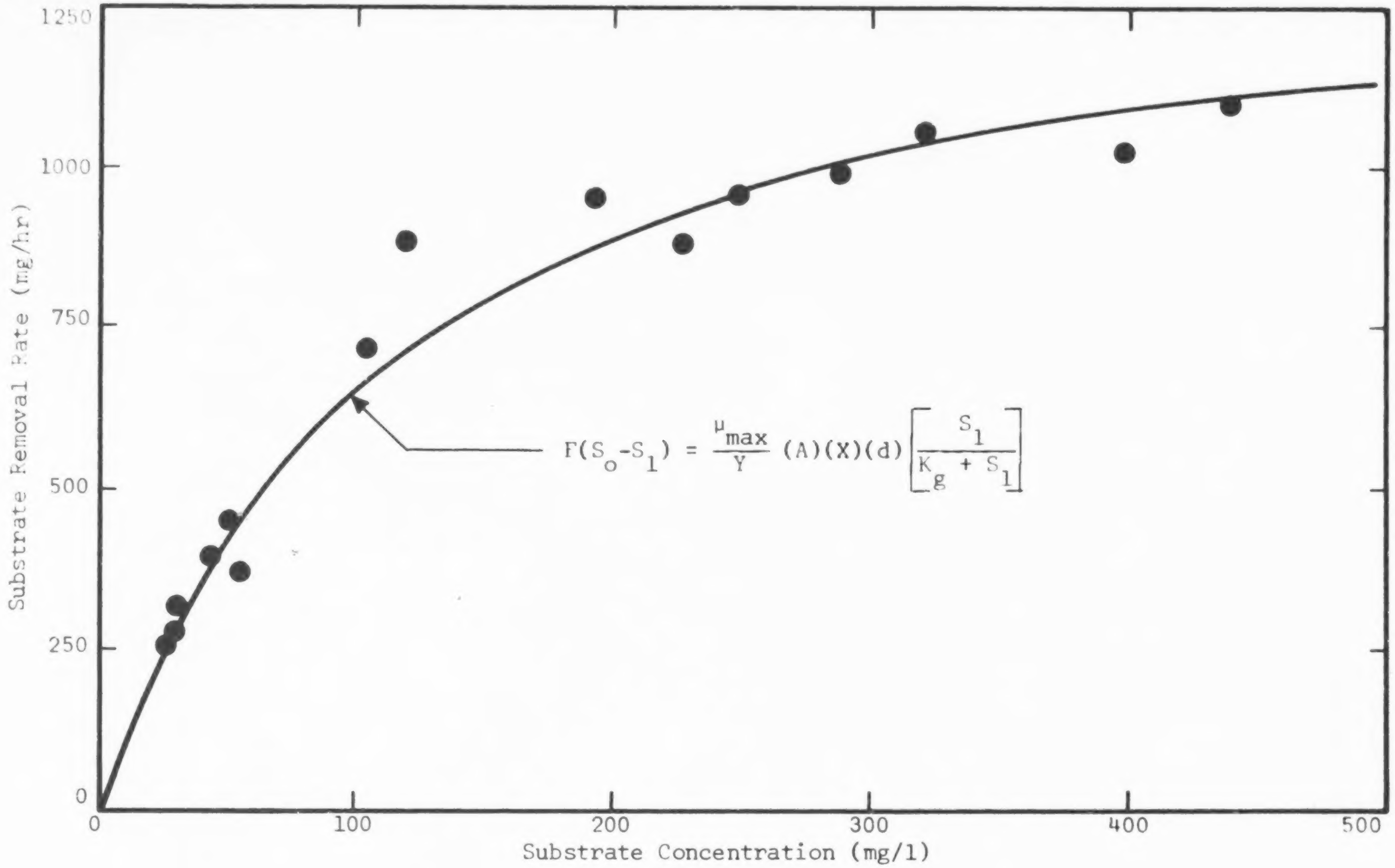

FIGURE 27. THE EFFECT OF CONCENTRATION ON THE RATE OF SUBSTRATE REMOVAL FROM SINGLE STAGE, FIXED FILM REACTORS

Third, the agreement found between the theoretical curve and observed results clearly supports the validity of the proposed model. One additional result of interest was the elemental analysis of the biological film. The carbon-hydrogen-nitrogen analysis of films developed at various substrate concentrations showed an average composition of 39.2 percent carbon, 6.11 percent hydrogen, 9.83 percent nitrogen and 44.9 percent oxygen. As shown in Table 5 there was some variation in cell composition at various substrate concentrations, but this was apparently a random occurrence since no meaningful relation could be found between film composition and substrate level. One interesting point was the nitrogen composition of the biological film. The average nitrogen content of 9.83 percent is below the value of 12 percent as given in the empirical formula $C_5H_7NO_2$ as reported by Hoover and Porges (112). The difference was apparently due to the presence of a polysaccharide matrix in the film mass. Based on the results of this experiment the biological film may be approximated by the empirical formula $C_9H_{17}N_2O_8$.

TABLE 5

BIOLOGICAL FILM COMPOSITION AT VARIOUS SUBSTRATE CONCENTRATIONS

Substrate Range (mg/l)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Oxygen[**] (%)
286-322	38.6	6.29	9.80	45.3
192-249	38.4	6.06	9.64	45.9
44-58	39.9	6.06	10.03	44.0
27-32	36.7	5.65	9.44	48.2
400-440	41.1	6.40	10.02	42.3
100-120	40.4	6.20	10.03	43.4
Average	39.2	6.11	9.83	44.9

[*] Samples were composite masses of film from three reactors during each run and the results are based on the volatile fraction (0.92) only.

[**] $\% O_2 = 100 - \% C + \% H_2 + \% N_2$.

VII. MULTISTAGE AND PLUG-FLOW REACTORS

GENERAL

Although single stage, completely-mixed reactors provide an excellent means of studying the kinetics of biological films, a single stage model would not be expected to approximate the trickling filter process. However, the kinetic principles presented in Chapter VI may be extended to other reactor systems in which the geometry, flow patterns and residence time distributions closely approximate those observed in actual filters. In this investigation a series of completely mixed reactors was used to test the validity of the plug-flow model. Before testing the theoretical plug-flow model it was necessary to:

(1) Determine the number of completely mixed reactors that would provide a reasonable approximation of the plug-flow model.

(2) Insure that the experimental reactor system had residence time distribution characteristics similar to the theoretical reactor system.

The first task was accomplished by comparing the theoretical substrate removal for a plug-flow reactor to that predicted for various numbers of completely mixed reactors in series. The second was accomplished through residence time distribution studies.

Following the selection of the experimental system studies were conducted to test the validity of the theoretical models.

SELECTION OF A REACTOR SYSTEM

From an analysis of the theoretical plug-flow and series reactor model it was determined that the substrate removal through six reactors was essentially equal to that predicted for the plug-flow model. This calculation was based on a plug-flow reactor in which the active microbial mass per unit depth was identical to that in each completely mixed reactor. This provided a basis for comparison. The theoretical substrate removal at various concentrations for the two systems is shown in Figure 28. A deviation between the two theoretical substrate removal values of 2-3 mg/l occurs in the influent concentration range of 100-200 mg/l, but diminishes on either side of this range and becomes indistinguishable as the concentration approaches either zero or 400 mg/l. It was decided that six completely mixed reactors in series would provide a valid approximation of a plug-flow system. However, it was still necessary to demonstrate that the six experimental reactors in series had residence time distribution characteristic similar to six, ideal, completely-mixed reactors.

RESIDENCE TIME DISTRIBUTION STUDIES

Procedure

The residence time distribution of the six annular reactors in series was studied by pulse injecting 5 ml of Rhodamine 6 GDN extra dye into the first reactor and continuously monitoring the effluent from the sixth with a Turner Fluorometer. Before injecting the dye the flow rate was adjusted to 800 ml/min and the temperature at 25°C. The flow through the reactors was water instead of substrate. This change was made to preclude organism growth and dye adsorption in the system.

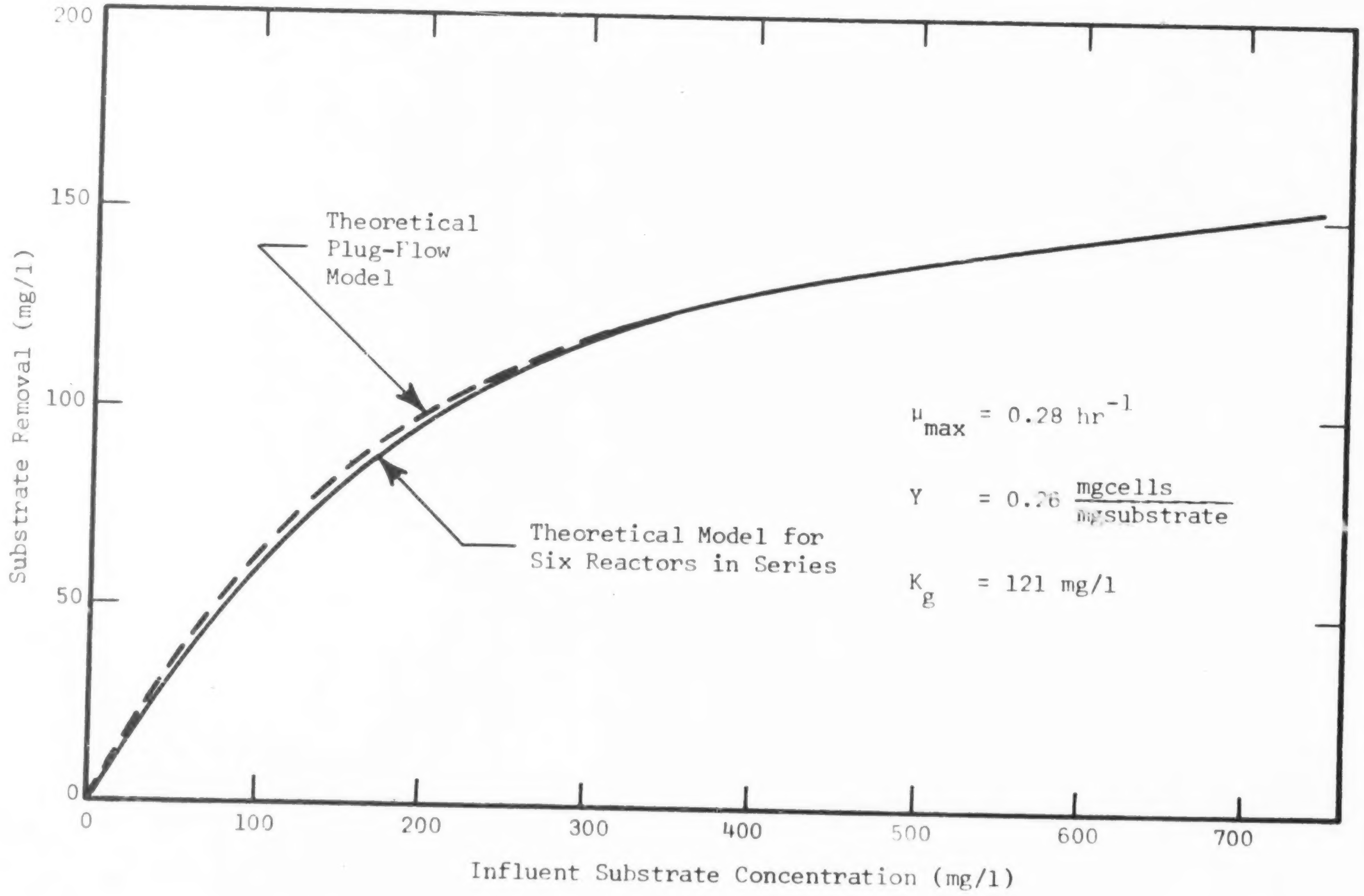

FIGURE 28. A COMPARISON OF THE THEORETICAL SUBSTRATE REMOVAL FOR PLUG-FLOW AND SERIES REACTOR MODELS

All other conditions were identical to those used for the single stage mixing studies described in Chapter V.

Results and Discussion

As shown in Figure 29 the residence time distribution curve for the experimental system was extremely close to that for six ideal completely mixed reactors. The peak of the observed distribution curve had a height of 0.98 on the reduced concentration scale compared to the theoretical value of 1.05. The observed distribution curve was also shifted to the right, with the peak occurring at a reduced time of 0.90 compared to 0.82 for the theoretical curve. The most probable reasons for the deviation between the observed and theoretical values are: (1) a slight departure from ideal complete mixing in each of the six individual reactors and (2) the volume of the connecting tubing between the reactors increased the actual residence time 1-2 percent above the theoretical value of 7.5 minutes. However, from a practical standpoint the magnitude of the deviation between the theoretical and observed residence time distribution is insignificant, and the assumption of complete mixing valid.

It was also of interest to compare the distribution curve for six completely mixed reactors to that of ideal plug-flow systems and actual filters.

The residence time distribution curves shown in Figure 30 for packed towers were calculated from the data of Eckenfelder and Barnhart (101) and Sinkoff et al. (34). The solid curve represents the calculated residence time distribution of an 18 ft. Polygrid filter loaded at a hydraulic rate of 8.0 gpm/ft^2. The dashed curve was calculated from the data of Sinkoff et al. (34) and represents a column

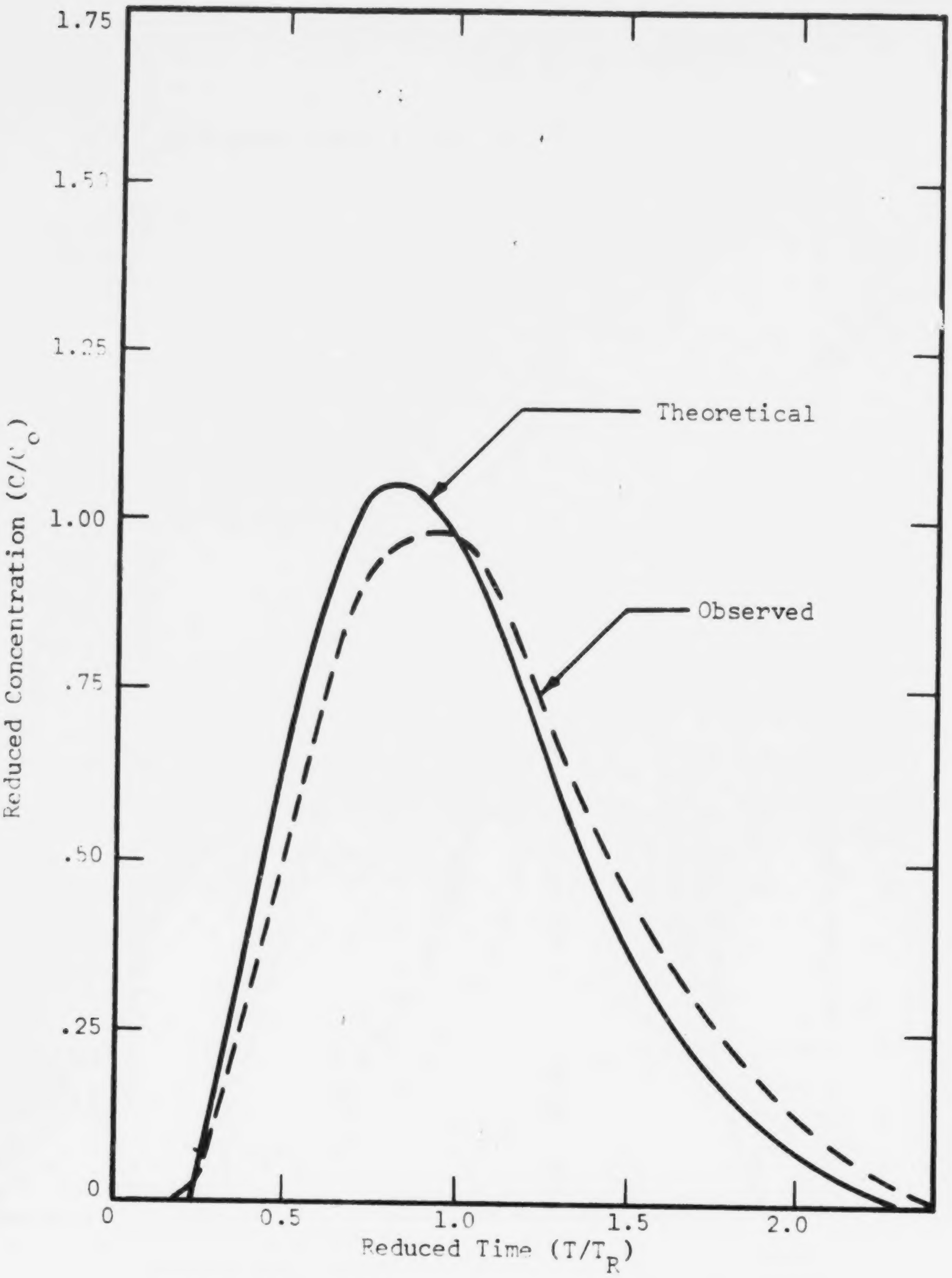

FIGURE 29. RESIDENCE TIME DISTRIBUTION CURVES FOR SIX REACTORS IN SERIES

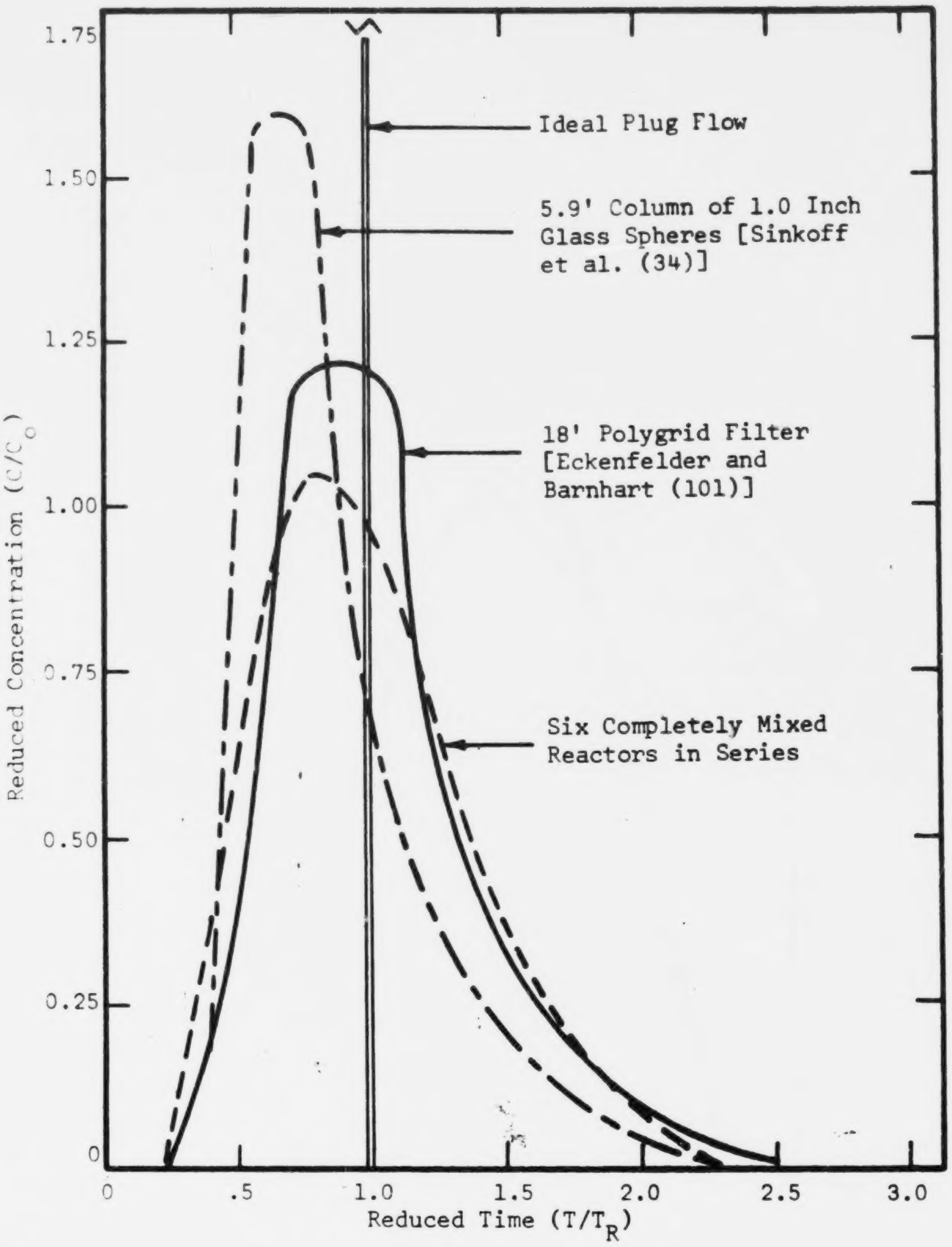

FIGURE 30. A COMPARISON OF THE RESIDENCE TIME DISTRIBUTION CURVES FOR TRICKLING FILTERS WITH TWO THEORETICAL SYSTEMS

5.9 ft. deep with 1-inch glass sphere packing and loaded at 1.06 gpm/sq ft. Sodium chloride solutions were used as the tracer in both investigations and the centroid of the concentration-time curve was used as the basis for determining the average residence time.

The flow through both filters more closely approximated ideal plug-flow conditions than six reactors in series, and exhibited the near normal distribution reported by Danckwerts (100) for packed towers. Danckwerts also noted that ideal plug-flow reaction models usually describe packed towers quite well in spite of this deviation from ideal plug-flow. In this case, it has been shown that the substrate removal through a system of six reactors in series, which does not approximate plug-flow as well as packed towers, provides an adequate prediction of the substrate removal through a plug-flow reactor. Therefore, it appears that the trickling filter may be adequately described by a plug-flow model and approximated by the experimental system of six annular reactors in series if the equations are valid and steady-state may be assumed.

TRANSIENT RESPONSE

Procedure

The use of a steady-state equation for the design of waste treatment facilities is theoretically feasible only when the system has a rapid response to change. To test the response of a fixed-film system, step changes were made in the influent substrate concentration and the effluent concentration was determined at 7.5, 15, 22.5 and 30 minutes after initiating the change. This method provided a means of determining the time required for a system to go from one steady-state value to another. The observed results were also compared to the theoretical transient response equations which were solved using a digital-analog simulation program (S/360-CSMP) (115) on the IBM 360. The two step changes made were from 525 to 400 mg/l and from 400 to 145 mg/l.

Results and Discussion

Both the theoretical and observed results of the transient studies shown in Figure 31 demonstrate the rapid response of fixed-film reactors to changes in influent substrate concentrations. The theoretical curve of the effluent concentration revealed that new steady-state conditions should have been attained 18 minutes after making both the step decrease in influent concentration from 525 to 400 mg/l and from 400 to 145 mg/l. After 11.7 minutes the theoretical effluent concentrations were within 10 mg/l of the new steady-state values and approached to within 2 mg/l of the new steady-state concentration after only 15 minutes.

As shown in Figure 31 the observed and theoretical results are in good agreement. Following the step change in influent concentration from 525 to 400 mg/l the new steady-state concentration of 270 mg/l was rapidly approached. After 15 minutes the observed effluent concentration was down to 285 mg/l and decreased to 278 and 256 mg/l at 22.5 and 30 minutes, respectively.

The agreement found between the observed and theoretical results following a step change from 400 to 145 mg/l was encouraging. At 7.5 minutes after the step change the observed effluent concentration was 146 mg/l compared to a theoretical value of 145 mg/l. Fifteen minutes after the step change both the theoretical and observed effluent

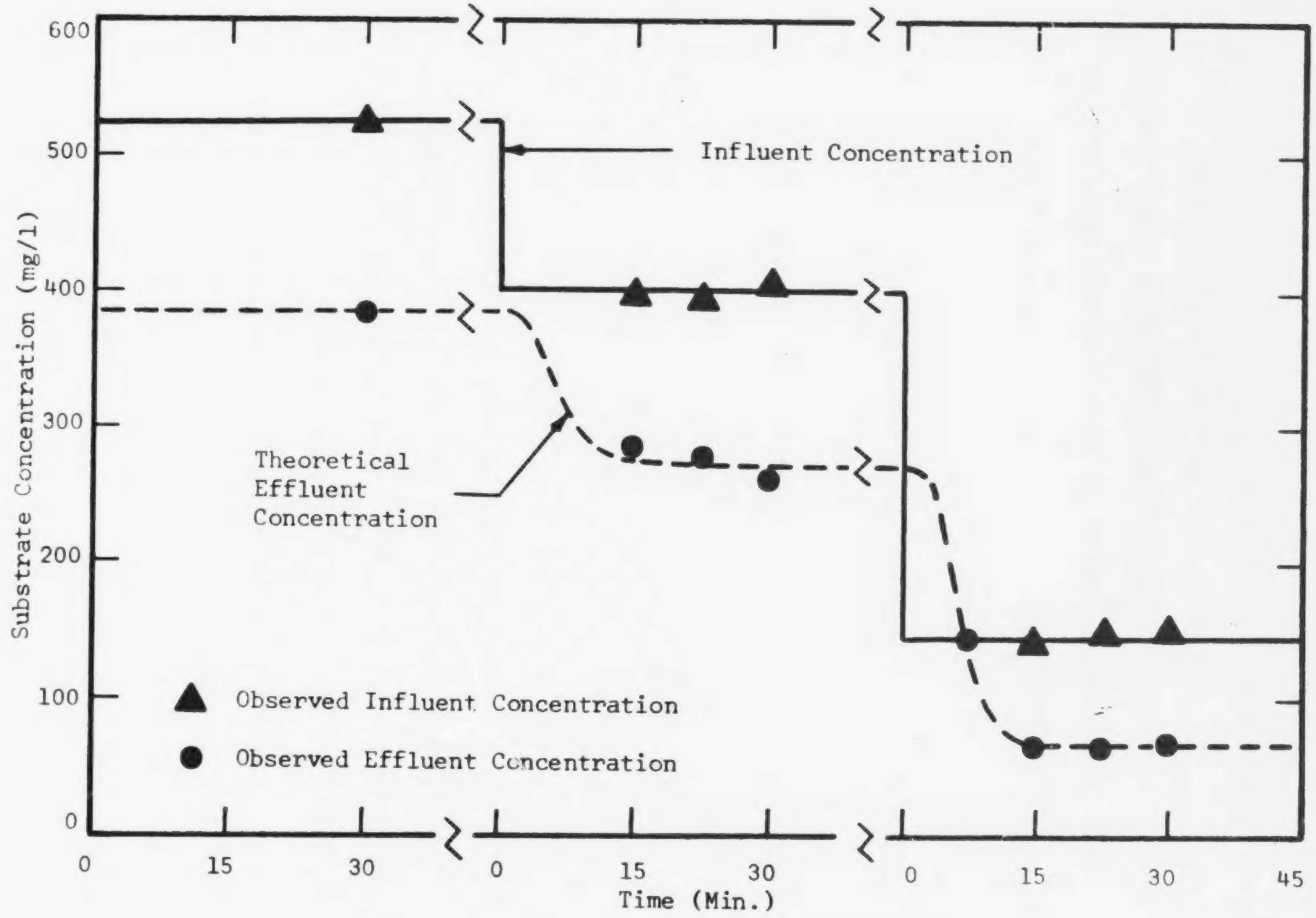

FIGURE 31. RESPONSE OF SIX FIXED-FILM REACTORS IN SERIES TO STEP CHANGES IN INFLUENT CONCENTRATION

concentration were 68 mg/l. The observed effluent concentration at 22.5 and 30 minutes after the step change was 62 and 66 mg/l, respectively, compared to the theoretical steady-state concentration of 66 mg/l.

In view of the rapid response of fixed-film biological reactors and the stabilizing effect provided by primary treatment vessels it is unlikely that serious error would be introduced by using a steady-state design equation for trickling filters.

STEADY-STATE SUBSTRATE REMOVAL

Procedure

Although total substrate removal values were obtained at influent substrate concentrations of 525, 400 and 145 mg/l during the transient studies, it was also desirable to determine the removal in each reactor throughout the system. Therefore, duplicate steady-state substrate values were determined for each of the six reactors at average influent concentrations of 746, 273, and 100 mg/l. This information provided a means of approximating the rate of substrate removal with depth in a plug-flow reactor, or in a series of completely mixed reactors. Furthermore, the use of the steady-state removal values observed at the six influent concentrations provided a means of comparing the observed and theoretical substrate removal as a function of influent concentration.

During these tests the mixing speed was 100 rpm, the pH 6.5 ± 0.1 and the temperature was maintained at 25 ± 0.5°C. The flow for these multistage tests was increased from the 134 ml/min used for the single stage test to 800 ml/min to retain a total retention time in the system of 7.5 min.

The reactors were innoculated with domestic sewage as outlined in Chapter V and operated under batch conditions until a thin film of organisms developed on the reactor walls. The flow was then gradually increased to 800 ml/min and the influent substrate concentration adjusted to approximately 746 mg/l of glucose. After the reactors had reached steady-state conditions as indicated by constant dissolved oxygen and substrate concentrations, the six reactors were sampled in reverse order. The second duplicate sample was taken after an additional hour of operation. The process was repeated at influent concentration of 273 and 100 mg/l.

All glucose concentrations were determined in triplicate with the Technicon Autoanalyzer as described in Chapter IV. Methods were not available to determine the high dissolved oxygen influent concentration (>50 mg/l), and due to the problems encountered in achieving a constant film thickness in all six reactors, total and volatile suspended solids were not obtained. However, carbon-hydrogen-nitrogen analysis and microscopic examinations were made of the films in each reactor in order to detect any major change in the characteristics of the microbial population through the reactors.

Results and Discussion

The substrate profile through the six annular reactors closely followed the theoretical values as shown in Figures 32, 33 and 34. Figure 32 presents the observed substrate concentration in each of six reactors for an average influent concentration of 746 mg/l as well as the theoretically predicted values for reactors in series and plug-flow

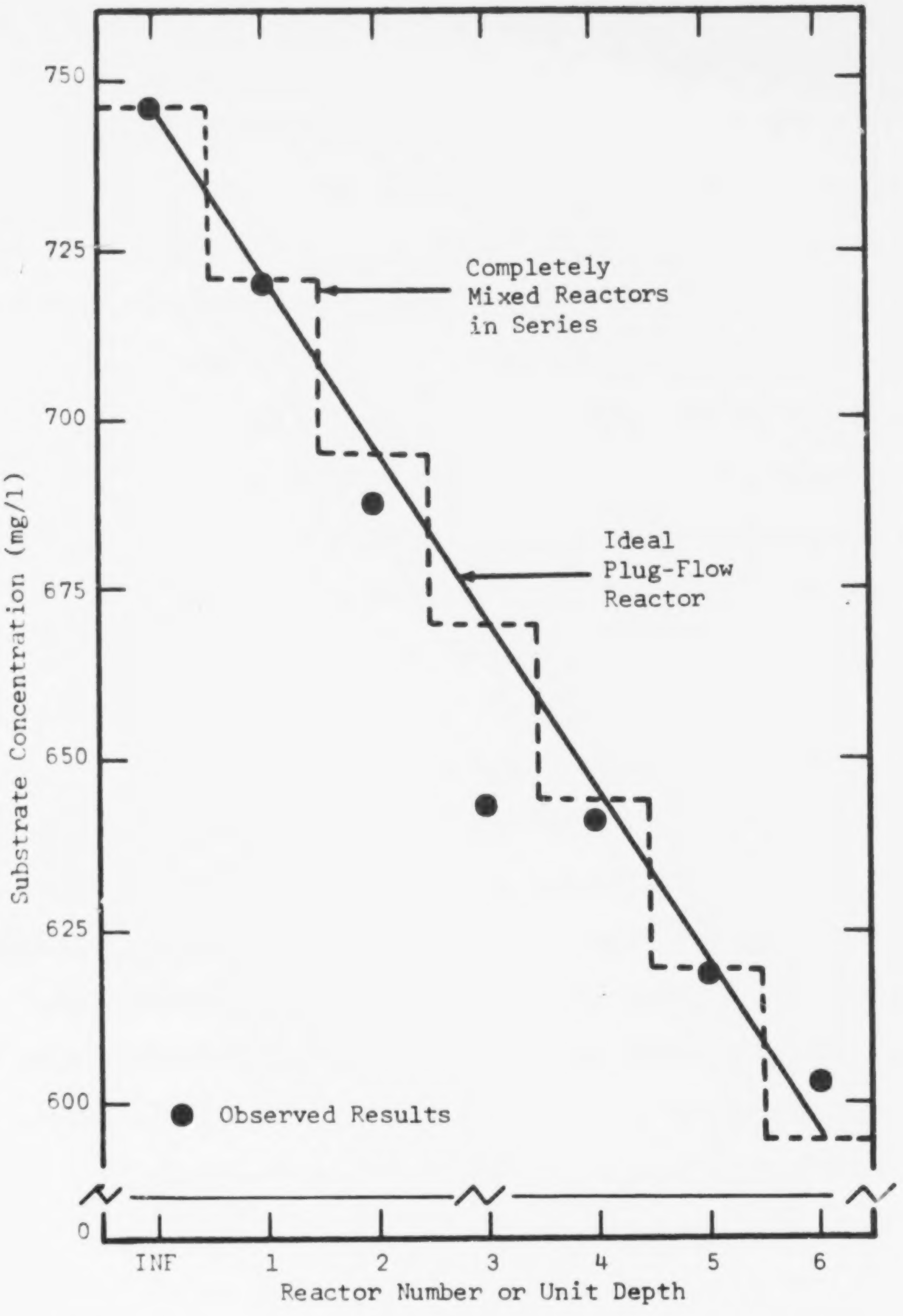

FIGURE 32. A COMPARISON OF THE THEORETICAL AND OBSERVED SUBSTRATE PROFILE FOR AN INFLUENT CONCENTRATION OF 746 MG/L

systems. As expected, the removal through the system at this high substrate concentration was nearly linear with a glucose removal of 26 mg/l in the first vessel and 25 mg/l in the sixth.

The predicted effluent substrate concentration was 594 mg/l for the plug-flow model compared to 595 mg/l for the series reactor model. Both predicted values were slightly below the average observed effluent concentration of 603 mg/l. In addition, the average observed substrate concentration of 643 in the third reactor was considerably lower than the predicted value of 670 mg/l for the theoretical models. The probable source of error was in sampling. The resistance through the sample lines was less than that through the reactor system so the dilution water flow tended to increase during sampling. This diluted the substrate and produced erroneously low results. In spite of the slight deviations noted, the predicted and observed results are in good agreement.

The substrate concentration profile through the six reactors shown in Figure 33 is for an average influent concentration of 273 mg/l, and compares the observed results with the theoretical substrate concentrations of plug-flow and series reactors. Here it should be noted that the substrate removal is beginning to deviate from linearity. The theoretical removal through the first reactor was 20 mg/l for both the plug-flow and series reactor model while the corresponding removal through the sixth was 17 mg/l. The predicted effluent concentration was 159 mg/l for the plug-flow model and 160 mg/l for the reactor in series model. The predicted effluent concentration of 159 mg/l for the plug-flow model was only 0.8 percent above the observed effluent concentration of 158 mg/l. In general, the observed

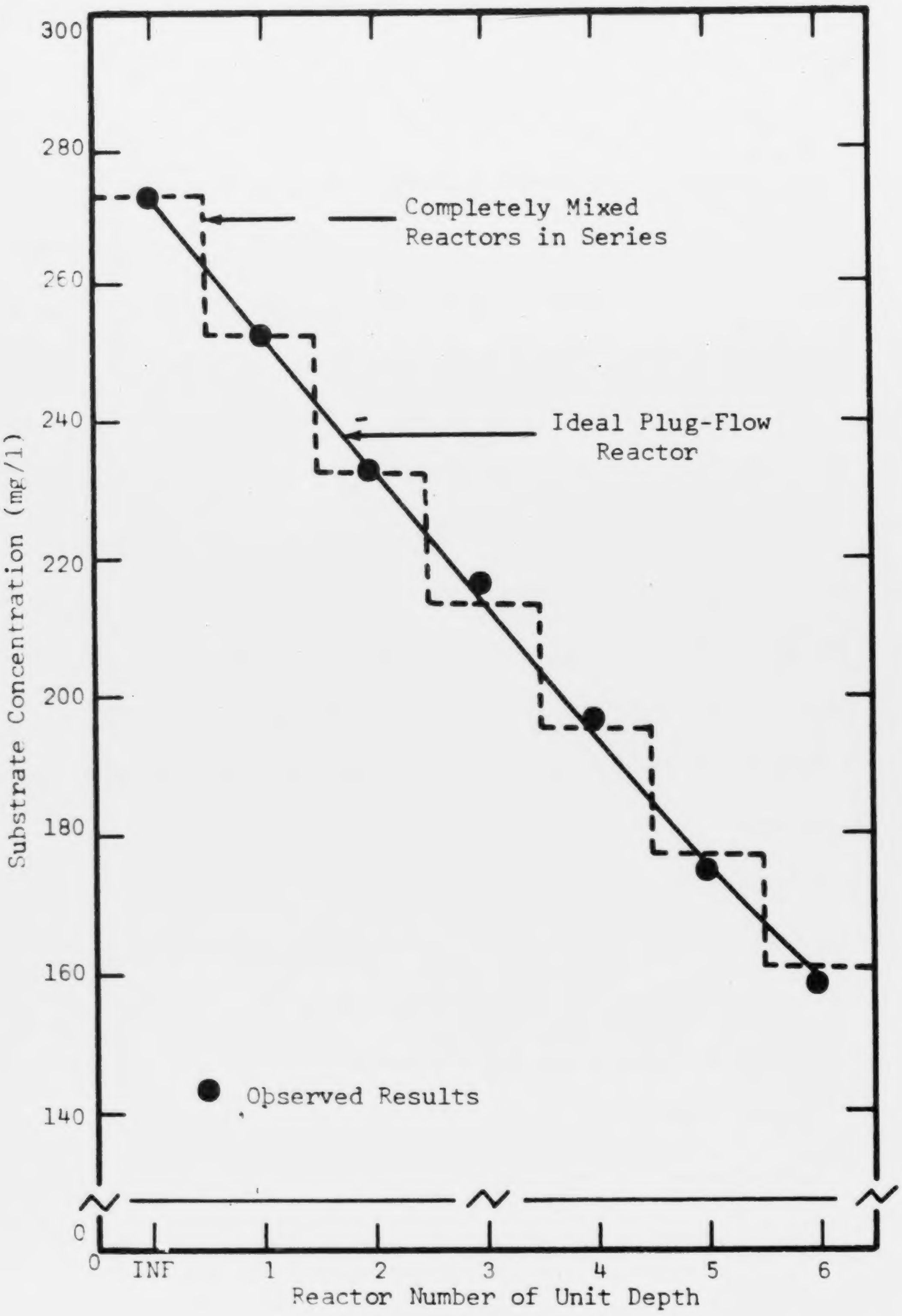

FIGURE 33. A COMPARISON OF THE THEORETICAL AND OBSERVED SUBSTRATE PROFILE FOR AN INFLUENT CONCENTRATION OF 273 MG/L

and theoretical substrate profiles for an influent concentration of 273 mg/l were in exceelent agreement.

A close examination of Figure 34 discloses two facts about the substrate profile for an influent concentration of 100 mg/l that are of interest. First, there was a slight deviation between the substrate concentration for the plug-flow and reactor in series model. However, the difference of only 2.0 mg/l between the predicted effluent of the plug-flow model of 38 mg/l and the reactors in series model of 40 mg/l is still relatively small. Another important fact is that there is a significant difference in the theoretical substrate removal through each reactor. The predicted removal decreased from 13 mg/l in the first reactor to 7 mg/l in the sixth by the series reactor model and from 13 to 8 mg/l for the corresponding reactors by the plug-flow model. In addition, the agreement between the observed and two theoretical profiles is rather poor. The observed substrate removal through the system was 11 percent greater than that predicted by the plug-flow model and 15 percent above the predicted value of the series reactor model. The errors, however, appear to be due to dilution as previously mentioned.

A better comparison of the observed results and the two theoretical models may be obtained from a plot of influent substrate concentration vs. substrate removal as shown in Figure 35. The data at influent concentrations of 525 mg/l, 400 mg/l, and 145 mg/l represent the average steady-state results obtained before and after each transient response study. The information for influent concentrations of 746, 273 and 100 mg/l represent the influent and effluent of the steady-state profile studies just described.

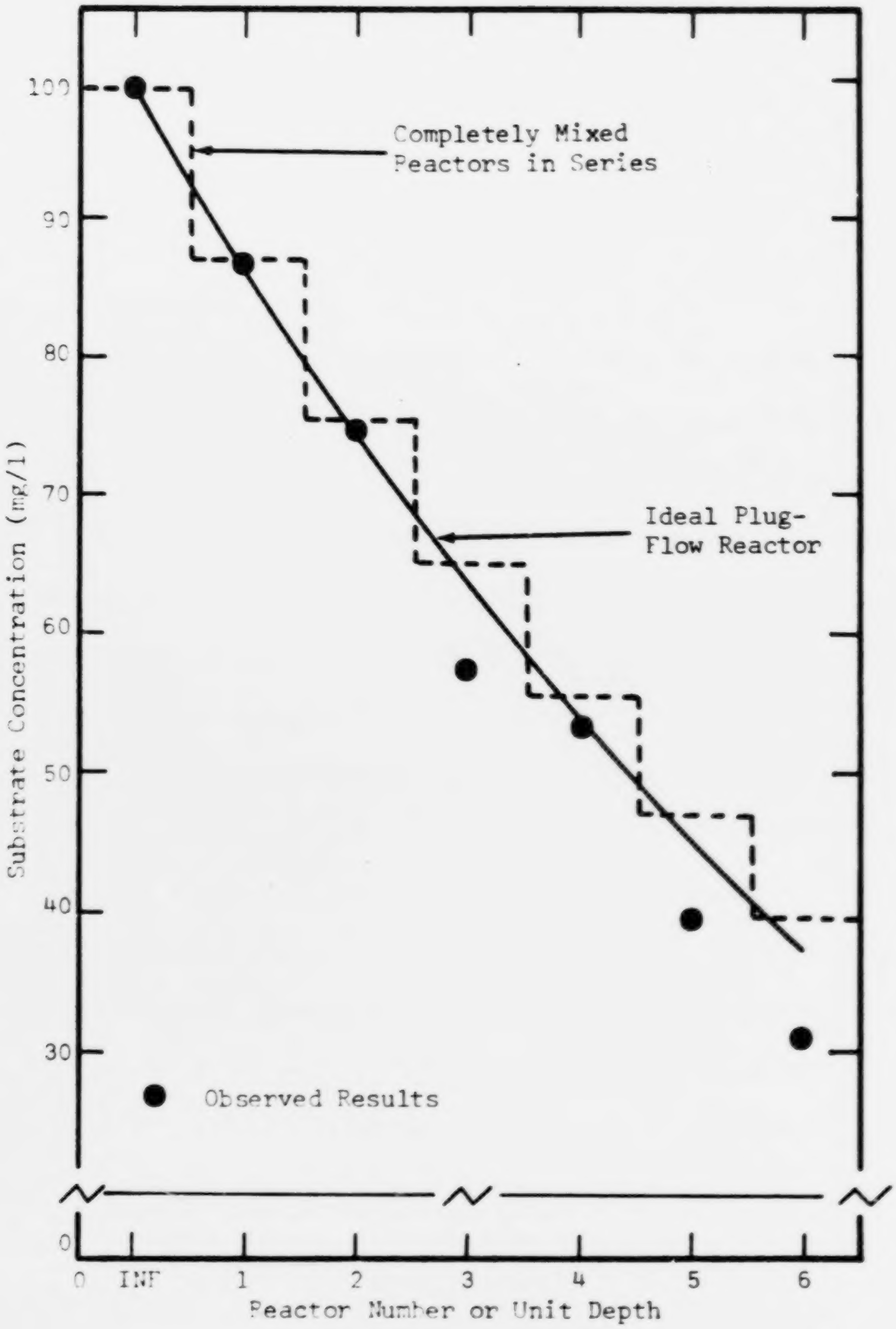

FIGURE 34. A COMPARISON OF THE THEORETICAL AND OBSERVED SUBSTRATE PROFILE FOR AN INFLUENT CONCENTRATION OF 100 MG/L

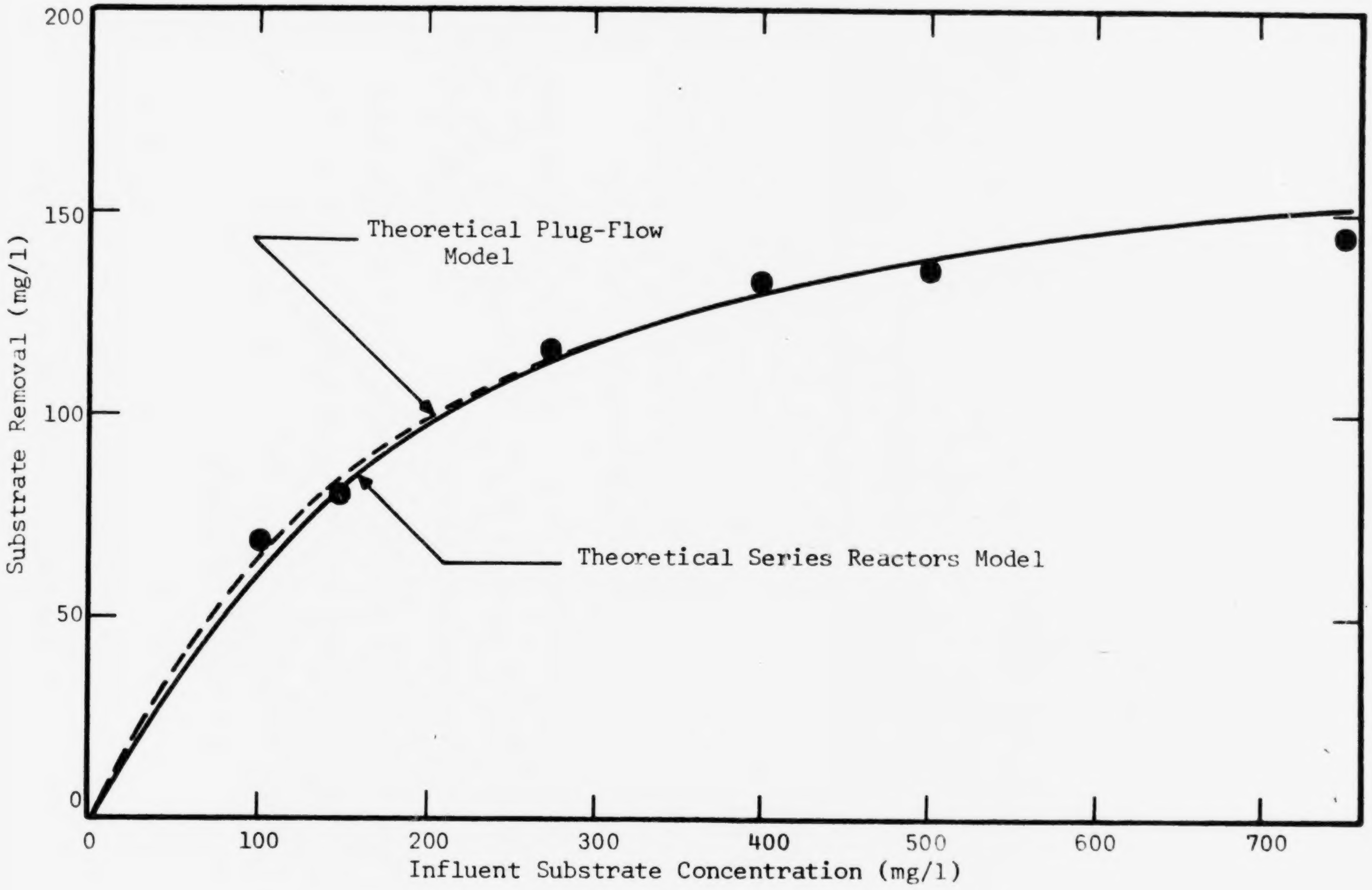

FIGURE 35. THE EFFECT OF INFLUENT CONCENTRATION ON SUBSTRATE REMOVAL FOR SIX REACTORS IN SERIES

Once again it is important to note that the plug-flow model

$$(S_o - S_e) = \frac{\mu_{max}}{FY}(a)(X)(d)(H)Z - K_g \ln \frac{S_o}{S_e} \qquad (70)$$

and the series reactor model

$$(S_o - S_n) = \frac{\mu_{max}}{FY}(A)(X)(d) \sum_{n=0}^{n=6} \left[\frac{S}{K_g + S}\right] \qquad (65)$$

predict essentially the same effluent substrate concentration for various influent concentrations. In addition the observed results show good agreement with both theoretical models. In fact the correlation coefficient between the observed results and theoretically predicted values of the plug-flow model was 0.96. This offers firm support for the validity of the theoretical model and it will be shown in the following chapter that the theoretical plug-flow model explains many of the phenomena previously observed in trickling filters.

Based on a carbon-hydrogen-nitrogen analysis run on the biological film from each of the six series reactors there was no systematic trend in cell composition to indicate a change in storage products or nutrient utilization in the various reactors. This is shown in Table 6 which presents the average of triplicate carbon-hydrogen-nitrogen analysis run of biological film samples from each of the six reactors. Although there was some variation in the cell composition, no significant correlation was found at the 95 percent confidence limit between reactor position and either carbon or nitrogen content. The average nitrogen content during these tests was 9.9

percent compared to 9.8 percent during the single stage test. As before, the low nitrogen content was probably due to the gelatinous polysacchride matrix of the biological film.

The microscopic examination of gram-stain preparations revealed that the major portion of the organisms in each reactor were gram-negative rods. The remaining fraction contained both gram positive rods and cocci, but no filaminteous bacteria were observed. The gram negative rods were approximately 0.5 - 1.0 μ in width and 2 - 3 μ in length. The gram positive rods were somewhat larger. There was no detectable change in the film characteristics through the series reactor system from either gram stains or microscopic examinations.

TABLE 6

CARBON-HYDROGEN-NITROGEN ANALYSIS FOR
BIOLOGICAL FILM FOR SIX SERIES REACTORS

	Cell Composition*			
Reactor Number	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Oxygen** (%)
1	39.8	7.45	9.60	43.2
2	41.7	7.46	10.02	40.8
3	45.4	7.75	9.70	37.2
4	42.7	7.72	9.80	39.9
5	44.4	7.57	10.50	37.5
6	42.7	7.66	10.10	39.5
Average	42.8	7.60	9.95	39.5

*Based on the Volatile Fraction only (Corrected for 10% Ash).

**Calculated by 100% - (% C + % H + % N).

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VIII. ENGINEERING APPLICATIONS AND SIGNIFICANCE

GENERAL

Mathematical models based on the theory of continuous culture of microorganisms provide an insight into the mechanism of organic waste utilization by the trickling filter that has not been previously available. However, the equations also contain a number of elusive kinetic constants which may lead to either "misuse" or "disuse" by the practicing engineer. It is quite possible that such constants as yield, maximum specific growth rate, unit mass of biological film and active film thickness need not be determined on an individual basis for design applications. These parameters may be combined into a single term for simplification, but their presence and significance should be thoroughly understood.

APPLICATIONS

As previously mentioned, the kinetic parameters in the plug-flow model

$$(S_o - S_e) + K_g \ln S_o/S_e = \frac{\mu_{max}}{FY}(a)(X)(d)(H)Z \qquad (52)$$

would be quite tedious to determine on an individual basis. However, it should be possible to evaluate the product $\frac{1}{Y}\mu_{max}(X)(d)$ from pilot studies. This would suggest that a single term P be substituted into Equation 52 such that

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$$P = \frac{1}{Y}(\mu_{max})(X)(d) \qquad (71)$$

Equation 52 would then become

$$(S_o - S_e) + K_g \ln S_o/S_e = \frac{P}{F}(a)(H)Z \qquad (72)$$

The parameters included in the term P should be constant for a given set of environmental factors, and may be determined for the region of interest. It was shown in Chapter 5 that the active film thickness did not vary with mixing speed over the range of 50 to 200 rpm. Although not previously reported, the unit mass of the biological film was also constant over this range. This is demonstrated in Figure 36, where the relation between film thickness and wet film weight is shown. The film thickness was measured by microscopic examination and the wet film weight determined from removable slide weights after 5 minutes of draining. The slope of the curve represents the mass of wet film per unit area of slide, and was essentially equal for mixing speeds of 50, 100, 150 and 200 rpm. This indicates that the unit mass of film is not materially affected by mixing speed and P should be constant under the same environmental conditions.

With the value of P known the design engineer may then select the flow rate "F", the depth "Z", cross sectional area "H", and specific surface area of the filter media "a" which will most efficiently reduce the influent concentration S_o to the desired effluent S_e. It must be remembered that the saturation constant K_g varies with velocity and must be evaluated at each flow rate.

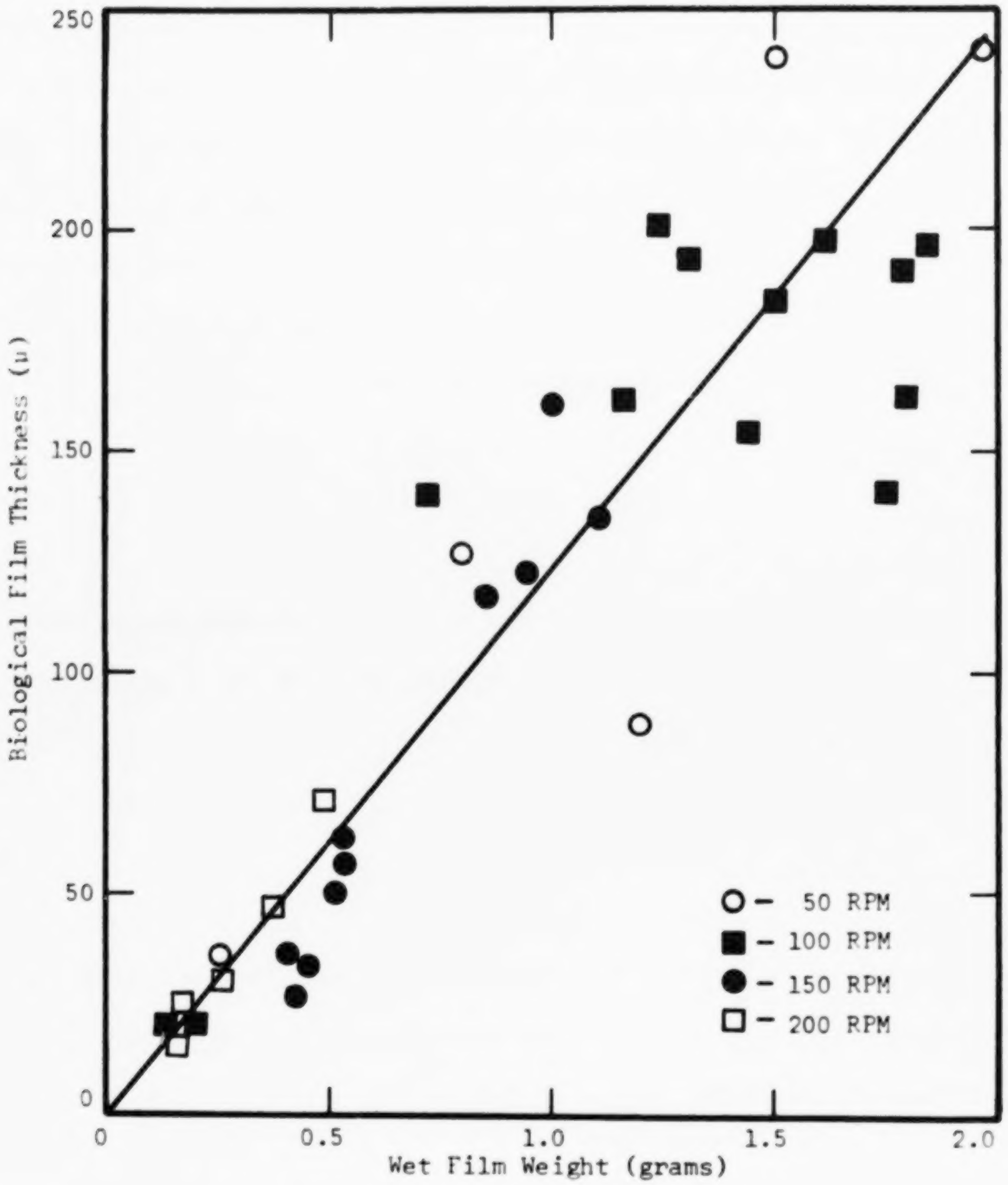

FIGURE 36. THE RELATION BETWEEN WET FILM WEIGHT AND FILM THICKNESS AT VARIOUS MIXING SPEEDS

The value of the constant P may be evaluated in several ways. To obtain a rapid estimate of P, apply the waste to be treated to a pilot filter having a known depth Z, and cross sectional area H. The specific surface area of the wetted filter media must be known. Whenever possible, increase the influent concentration S_o, or decrease the depth Z until the ratio S_o/S_e approaches one. As this occurs the value of $K_g \ln S_o/S_e$ will become insignificant and

$$S_o - S_e \cong \frac{P}{F}(a)(H)(Z) \tag{73}$$

From a knowledge of the influent and effluent substrate concentration, flow rate, and filter dimensions the value of P may be calculated from the approximate expression

$$P \cong \frac{(S_o - S_e)F}{(a)(H)(Z)} \tag{74}$$

The process may be more easily understood from an examination of the differential form of the mathematical model. It may be seen that when $S_e >>> K_g$ the equation

$$\frac{dS}{dZ} = \frac{\mu_{max}}{FY}(a)(X)(d)(H)\left[\frac{S}{K_g + S}\right] \tag{50}$$

reduces to

$$\frac{dS}{dZ} = \frac{\mu_{max}}{FY}(a)(X)(d)(H)(1) \tag{75}$$

since

$$\left[\frac{S}{K_g + S}\right] \approx 1 \tag{66}$$

By proper substitution Equation 75 becomes

$$\frac{dS}{dZ} = \frac{P}{F}(a)(H) \tag{76}$$

Here it is obvious that the rate of substrate removal with depth is constant and P may be estimated from the expression

$$P = \frac{\Delta SF}{\Delta Z(a)(H)} \tag{77}$$

With the value of P known the saturation constant K_g may be calculated for the flow rate employed.

The above method only provides estimated values of P and K_g, and is intended for use in the treatment of highly concentrated wastes. However, more accurate techniques of evaluation are available. Equation 72 may be written

$$S_o - S_e = P/F\ (a)(H)(Z) - K_g \ln S_o/S_e \tag{78}$$

which is similar in form to

$$Y = a + bm \tag{79}$$

Therefore a plot of (S_o-S_e) versus $\ln S_o/S_e$ should produce a line with negative slope K_g and intercept P/F (A)(H)(Z) at $S_o/S_e = 1$. For ease of presentation on semi-logarithmic paper the value $K_g' \log_{10} S_o/S_e$ may be substituted for $K_g \ln S_o/S_e$, where $K_g = 2.303\ K_g'$. Such a presentation is shown in Figure 37 for the experimental data obtained during the series reactor runs of Chapter VII. The data for six unit depths (Z = 6) represents the total substrate removal for both the steady-state and transient response studies. The information for unit depths of one and four were obtained from the steady-state studies in the first and fourth reactors of the series studies, respectively. Based on the observed substrate removal values from the single stage studies the product $\frac{P}{F}$ (a)(H)(Z) at unit depths of one, four and six should have been 29.8, 119.5 and 179 mg/l, respectively. The observed intercepts were 30, 120 and 160 mg/l.

The product P/F (a)(H)(Z) represents the maximum substrate removal that may be expected from a filter having a depth Z, cross sectional area H, and filter media with a specific surface area a. It may therefore be considered the maximum filter capacity for the flow rate F. The term P would then represent the capacity constant for a unit area of filter media. In this case the area capacity constant P was 0.72 mg/l per sq. cm. for unit depths of one and four and 0.64 mg/hr per sq. cm. at a unit depth of six.

The slope of the lines shown in Figure 37 represents the negative value of K_g', or the saturation constant for use with logs to the base 10. From these slopes it was calculated that the value of K_g' was 252, 230 and 184 mg/l for unit depths of one, four and six, respectively. The corresponding values of the saturation constant K_g for

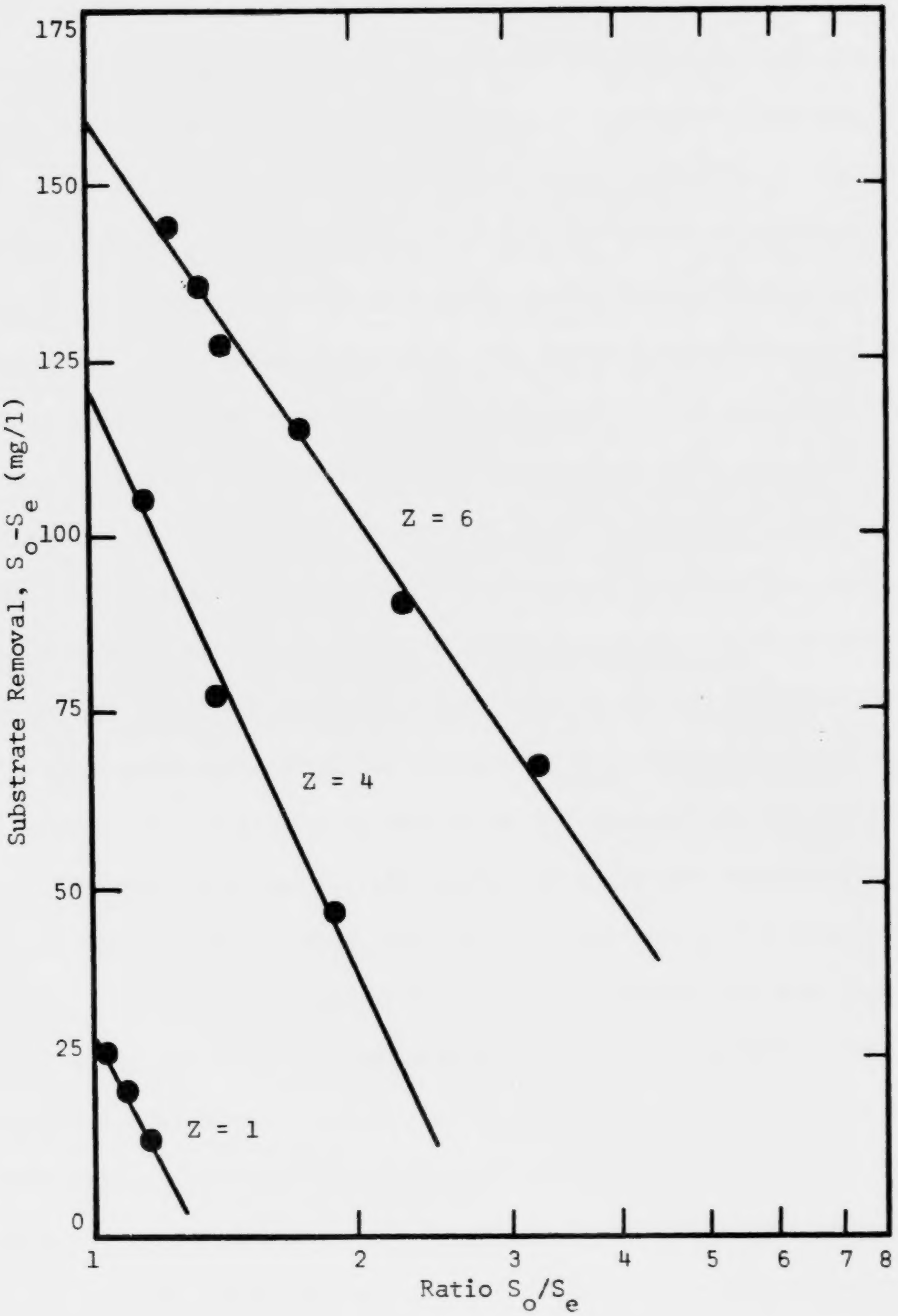

FIGURE 37. A GRAPHICAL DETERMINATION OF THE AREA CAPACITY CONSTANT P AND THE SATURATION CONSTANT K_g

use with natural logs were 113, 100 and 80 mg/l. The values of 113 and 100 mg/l for unit depths of one and four, respectively, were in good agreement with the value of 121 mg/l determined for the saturation constant K_g in single stage studies. However, the value of 80 mg/l is considerably in error. A change in the value of K_g of this magnitude was totally unexpected since there was excellent agreement between the predicted substrate removal and observed results. This agreement was indicated by a correlation coefficient of 0.96 and was clearly depicted in Figure 35. This suggested that the predicted substrate removal may be insensitive to significant changes in the saturation constant when accompanied by small changes in the product P/F (a)(H)(Z). Therefore, the theoretical substrate removal was calculated for a system having a saturation constant of 80 mg/l and a maximum removal of 160 mg/l. This was then compared to the system having a saturation constant of 121 mg/l and the product P/F (a)(H)(Z) of 179 mg/l. The curves for the two systems are shown in Figure 38. As one would expect, the use of 80 mg/l for K_g and 160 mg/l for the product P/F (a)(H)(Z) would predict greater substrate removals at lower concentrations and lower substrate removals at high concentration. This is of course in agreement with the observed results. At influent substrate concentrations below 500 mg/l the difference between the two curves is less than 5 mg/l. As the influent concentration approaches infinity the difference between the two curves approaches the maximum deviation of 19 mg/l (179 mg/l - 160 mg/l). This demonstrates that the predicted substrate removal is relatively insensitive to significant changes in the saturation constant K_g when accompanied by small changes in the product P/F (a)(H)(Z). However, changes in the saturation constant K_g are

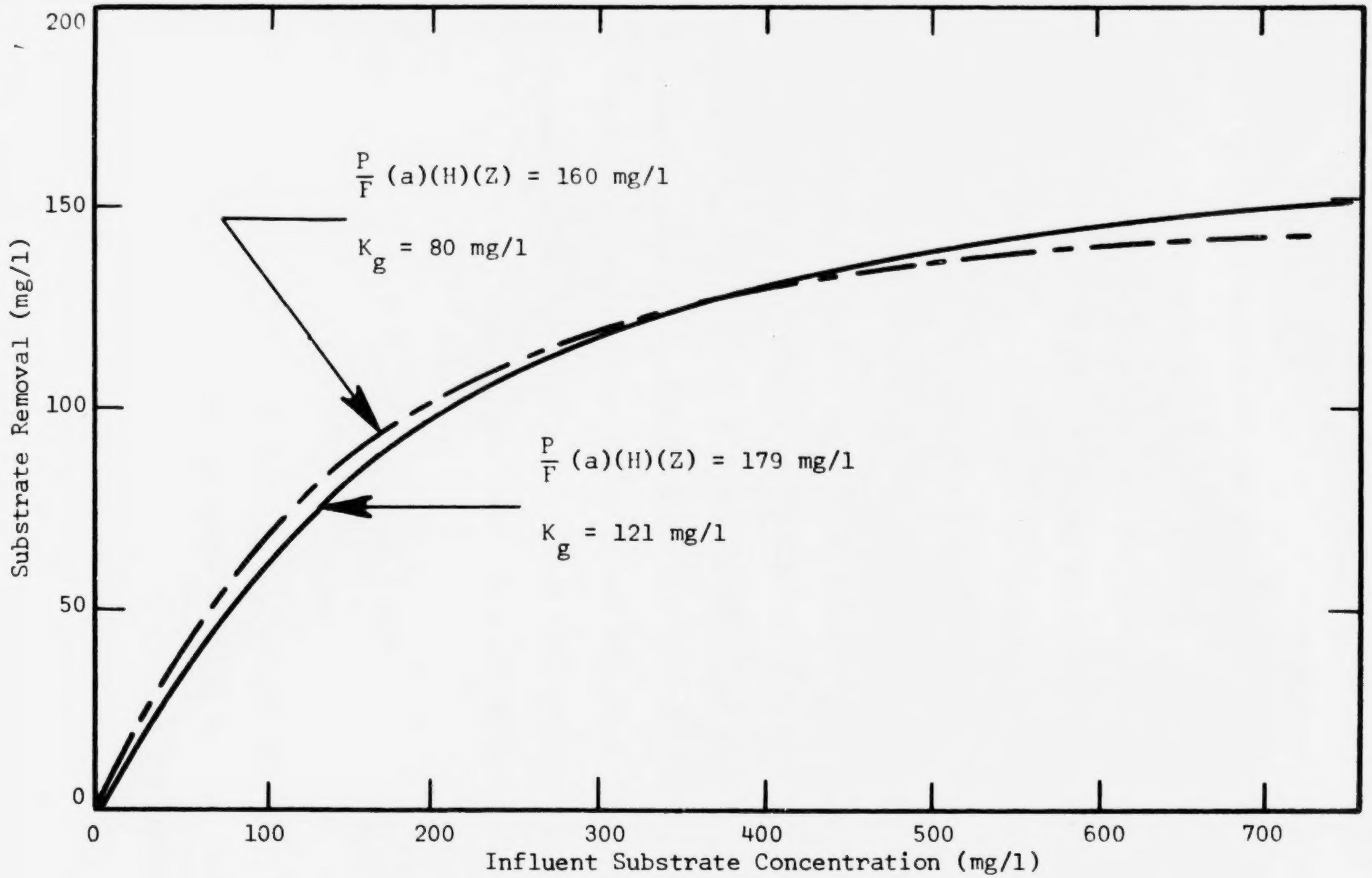

FIGURE 38. A DEMONSTRATION OF THE INSENSITIVITY OF THE SYSTEM TO SIGNIFICANT CHANGES IN K_g WHEN ACCOMPANIED BY SMALL CHANGES IN THE PRODUCT P/F (a)(H)(Z)

significant when the product P/F (a)(H)(Z) remains constant, as demonstrated later in this chapter.

When conducting the pilot test for the evaluation of the parameters P and K_g it would be important to vary the concentration over a wide range of values. Both very low and very high influent values should be employed, and the latter should be as high as possible. The closer the value S_o/S_e approaches one the better--since a minimum extrapolation would be required and the chance of error reduced.

The approximate method of evaluating P may be demonstrated using the series reactor data for an average influent concentration of 746 mg/l. The more closely the pilot unit approaches a differential reactor the closer the approximated value of P. Therefore, a unit depth of one will be used for the demonstration. The influent concentration to the unit depth was 746 mg/l, the effluent concentration 720 mg/l, and the removal was 26 mg/l. The value of the area capacity constant P calculated by the approximate expression

$$P = \frac{(S_o - S_e)F}{(a)(H)(Z)} \tag{74}$$

is then 0.61 mg/hr per sq. cm. This value of P is in fair agreement with the values of 0.72 and 0.64 mg/hr cm. sq. calculated by the graphical method.

SIGNIFICANCE

A realistic design process for the trickling filter must include a consideration of depth, cross sectional area, influent substrate concentration, flow rate (inflow and recirculation), rate of waste degradation and the presence of an active microbial film to accomplish this degradation. The necessity of a living microbial population for the removal of organic waste was demonstrated by Schlossing (19), and the importance of film thickness was reported by Sanders (56) and investigators in England (55). English investigators (80) have also found that filter performance is a direct function of the specific surface area of the filter media. Velz (31), Schulz (35), Eckenfelder (36) and others (32, 33, 38) have presented equations which express performance as a function of depth and flow rate. The proposed mathematical model

$$S_o - S_e = \frac{\mu_{max}}{FY} (d)(X)(a)(H)(Z) - K_g \ln \frac{S_o}{S_e} \qquad (52)$$

or

$$S_o - S_e = \frac{P}{F} (a)(H)(Z) - K_g \ln S_o/S_e \qquad (79)$$

includes all of these important parameters and explains many of the phenomena previously observed in trickling filters.

Beginning with the basic "law" of Velz (31) a number of models have been proposed which incorporate the first order model

$$- \frac{dL}{dD} = KL \qquad (5)$$

From an examination of the differential form of the proposed model

$$-\frac{dS}{dZ} = \frac{\mu_{max}}{FY}(d)(X)(A)(H)\left[\frac{S}{K_g + S}\right] \qquad (50)$$

it may be observed that a first order reaction is approached at low substrate concentrations. When the value of $S << K_g$ the denominator $(K_g + S)$ approaches a constant value K and Equation 50 approaches the first order form

$$-\frac{dS}{dZ} = \frac{\mu_{max}}{FY}(d)(X)(a)(H)\ \frac{S}{K} \qquad (80)$$

Therefore, at low substrate concentrations the proposed model would provide essentially the same results as the first order models. However, Equation 50 covers regions which the first order model will not describe. Both Velz (31) and Stack (32) postulated linear removal with depth at high substrate concentrations and this has been observed (95). In fact, it was demonstrated in the substrate profile for an influent concentration of 746 mg/l in Chapter VII. This region is covered by Equation 81. When the substrate concentration is much greater than K_g the term

$$\frac{S}{K_g + S} \simeq 1 \qquad (66)$$

and a linear rate of substrate removal with depth is predicted by the expression

$$-\frac{dS}{dZ} = \frac{\mu_{max}}{FY}(d)(X)(a)(H)(Z) \qquad (81)$$

In fact, the proposed model provides a continuous equation which describes the decreasing rate of organic waste removal from linear removal at high substrate concentration to essentially first order removal at low substrate concentrations. This corresponds closely to the retardant removal reported by Eckenfelder (36) and Howland et al. (37).

The proposed model also provides a possible explanation for the divergent effects reported for flow rate. It is generally recognized that the rate of organic waste removal decreases as $1/F^n$, but there is no general agreement on the value of n. Schultz (35) reported a value for n of 0.66, Swilley et al. (106) found the value to be greater than one and in an extensive investigation in England (113) values of 0.91 and 0.92 were reported. From the proposed model one would conclude that the effect of flow rate or velocity would vary, depending upon the region of operation.

In the equation

$$S_o - S_e = \frac{\mu_{max}}{FY}(d)(X)(a)(H)(Z) - K_g \ln S_o/S_e \qquad (52)$$

the effect of flow rate is manifest in two ways. First, the flow rate is expressed in the term F. Secondly, the saturation constant K_g is a function of velocity and therefore of flow rate. For very high influent substrate concentration the ratio S_o/S_e should approach one and the term $K_g \ln S_o/S_e$ should be negligible. The substrate removal should then vary as $1/F^1$. At lower substrate concentration, the apparent velocity effect would vary, and depend upon the relative substrate concentration as well as flow rate. This is due to the fact that K_g is a velocity dependent variable.

One point of interest that has not been mentioned thus far is filter efficiency as a function of organic loading. It was apparent from data presented in Chapters VI and VII that the rate of substrate removal increased with organic loading. However, the effect of organic loading on efficiency may not have been apparent. Therefore, the theoretical efficiency of a plug flow reactor as a function of influent concentration at constant flow rate is shown in Figure 39. The predicted efficiency decreases from 75 percent at an influent concentration of 10 mg/l to approximately 15 percent at an influent of 1000 mg/l. The absolute values are of little importance since they would change with the product P/F (a)(H)(Z) or K_g. However, the shape of the curve is of interest. There is very little change in filter efficiency at low influent concentrations, or organic loading rates. The efficiency then decreases rapidly with organic loading. This is similar to the observation on filter efficiency that led to the Ten State Standards (114).

The efficiency curve in Figure 39 is for any filter having a maximum capacity of 179 mg/l as represented by the producted P/F (a)(H)(Z) and a saturation constant K_g of 121 mg/l. This would appear to indicate that the efficiency would be independent of the relative dimension of the filter, as long as the maximum capacity constant P/F (a)(H)(Z) remained constant. However, this is not the case since the saturation constant K_g is a velocity dependent variable which decreases with velocity. This serves to explain some of the advantages of both recirculation and series filtration. The significance of the

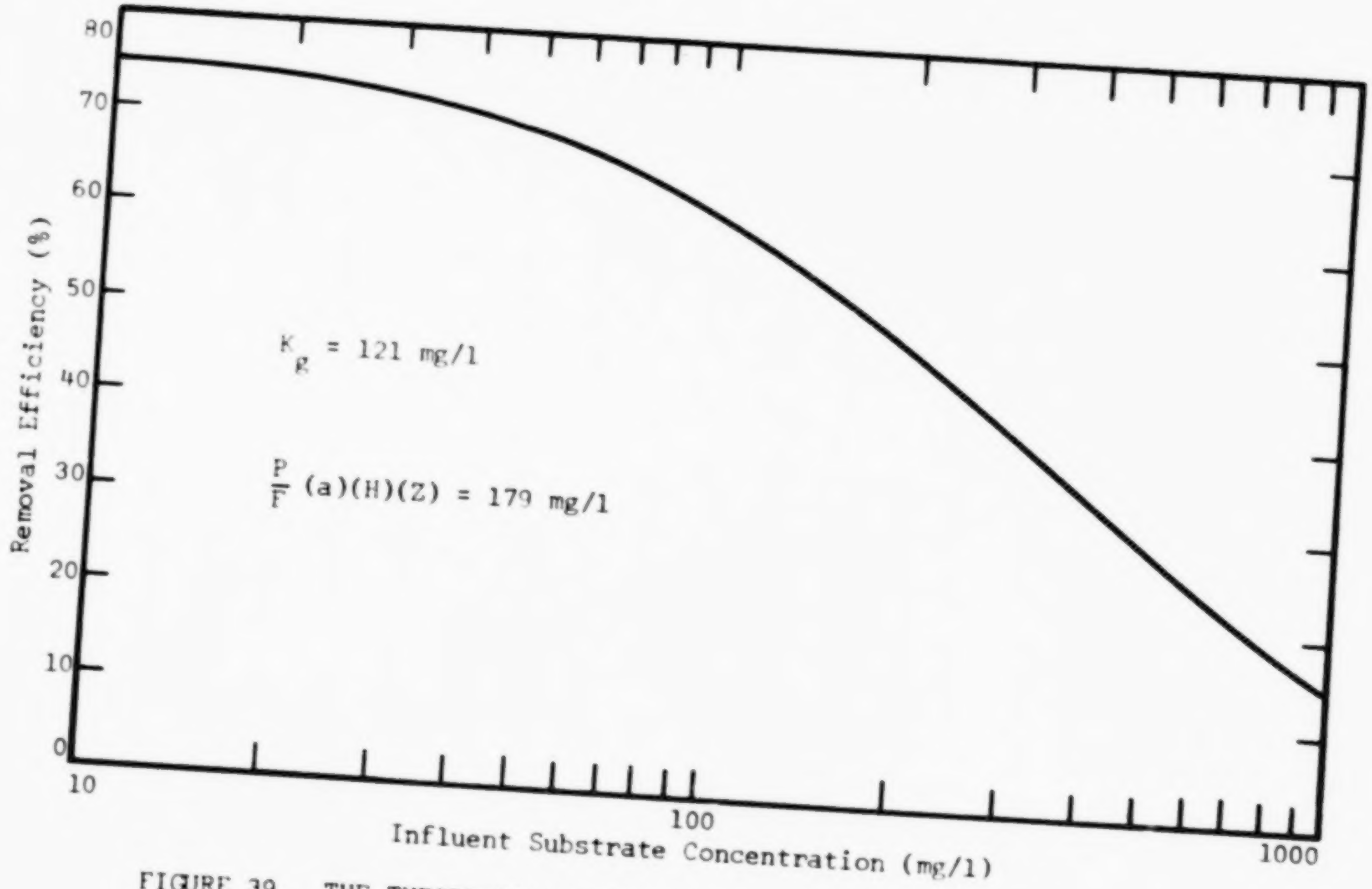

FIGURE 39. THE THEORETICAL EFFICIENCY OF A TRICKLING FILTER AS A FUNCTION OF THE INFLUENT SUBSTRATE CONCENTRATION

increase in liquid velocity provided by these methods of operations is shown in Figure 40. The substrate concentration obtained at various depths is shown for values of K_g of 75, 100 and 125 mg/l. For these calculations the influent concentration was held constant at 200 mg/l and the product P/F (a)(H) was assumed to be 30 mg/l per foot. The depth, Z, varied. Two points are apparent from Figure 40. First, the removal at any depth is greater for lower values of the saturation constant K_g. This would indicate a greater removal per unit volume of filter at higher surface loading rates since K_g decreases with velocity. It would, therefore, appear that filters should be proportioned so the recriprocal loading rate H/F is low and the depth Z is high. However, until the relation between K_g and hydraulic loading is explored further this can only be accomplished through pilot studies. The second point of interest is the rate of removal at the various depths. This follows the saturation phenomenon as expected and approaches a first order removal at low concentrations.

Although much remains to be done, it is felt that the proposed mathematical model provides an insight into the mechanism of organic waste removal by trickling filters that was not previously available. Design parameters such as depth, cross sectional area, flow rate and specific surface of the filter media are related to the "workman" population of the filter and the rate at which these organisms degrade organic waste. There are still a number of questions to be answered and tests to be made before the model may be generally applied, but it would appear that Equation 52 would serve as a rational foundation for trickling filter design.

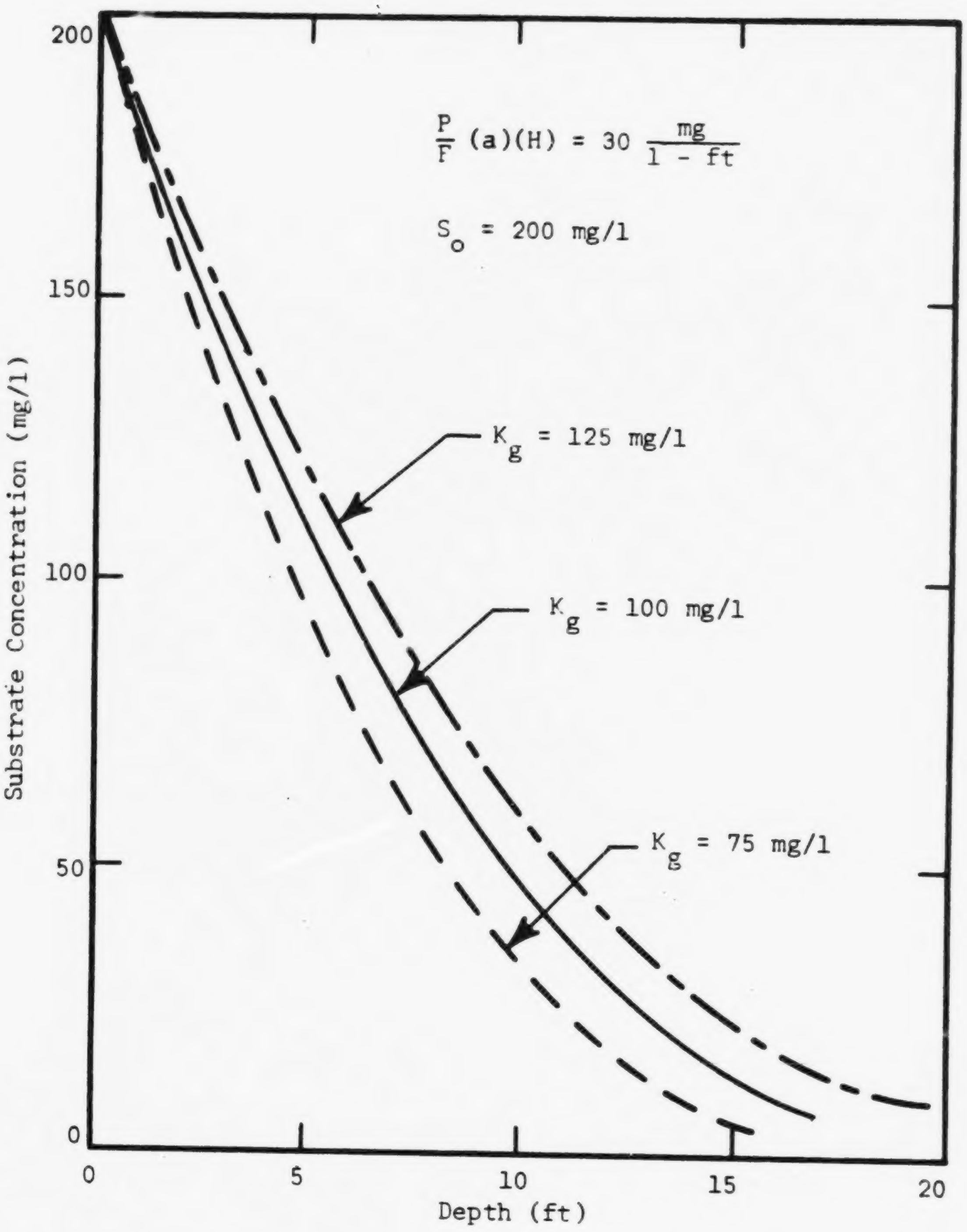

FIGURE 40. THE EFFECT OF THE SATURATION CONSTANT K_g ON THE PERFORMANCE OF FILTERS AT VARIOUS DEPTHS

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IX. CONCLUSIONS

During this investigation the kinetics of a mixed population of microorganisms were studied in both single and multistage reactors. The retention time of each system was maintained at 7.5 minutes so the suspended organisms were washed out and any substrate removal was due to the attached growth of microorganisms attached to the walls of the completely mixed, annular reactors. The study was conducted in three phases and the conclusions reached are presented in the same sequence in which they were conducted.

During the preliminary studies it was found that

1. The growth of biological films developed in three distinct phases. The first phase was logarithmetic and lasted until the active thickness was reached. During the second phase film growth was essentially linear and film continued to accumulate until the plateau film thickness was reached. This was the beginning of the third phase in which film accumulation was equal to zero and the newly formed cells were washed from the reactor.

2. Substrate utilization was proportional to the total film thickness until the active film thickness was reached but remained constant thereafter.

3. The active film thickness was independent of mixing speed while the plateau film thickness decreased with an increased mixing speed.

4. The active film thickness was 70 μ based on a weighted average of the data.

5. Substrate removal increased with mixing speed as a result of decreased resistance to diffusion by the liquid film.

6. The value of the saturation constant K_g incorporates the effect of liquid film diffusion and decreases with mixing speed.

7. A mixing speed of 100 rpm provided the most advantageous conditions for the remainder of the kinetic study. Mixing was essentially complete at this speed.

When three annular reactors were operated in parallel and under the same conditions to investigate the effect of dissolved oxygen and substrate concentrations it was found that:

1. The active film thickness was independent of the dissolved oxygen concentration when substrate controls the rate of biological growth.

2. Dissolved oxygen had no effect on the rate of substrate removal under the conditions of this study.

3. Substrate removal and organism growth increased with substrate concentration, but approached a maximum value. A saturation phenomena was definitely exhibited and may be expressed by

$$\mu = \mu_{max}\left[\frac{S}{K_g + S}\right]$$

4. Substrate removal in a completely mixed, fixed-film reactor may be described by the relationship

$$(S_o - S_e) = \frac{\mu_{max}}{FY}(A)(X)(d)\left[\frac{S_e}{K_g + S_e}\right]$$

5. The value of the saturation constant K_g was 121 mg/l the apparent yield, Y was 0.26 mg cell/mg substrate, and the maximum specific growth rate was 0.28 hrs^{-1} for the experimental conditions previously described.

From an analysis of the theoretical plug-flow and series reactor models it was found that

(1) The series reactor model

$$(S_o - S_n) = \frac{\mu_{max}}{FY}(A)(X)(d)\sum^{n=6}\left[\frac{S}{K_g + S}\right]$$

and plug-flow model

$$(S_o - S_e) + K_g \ln S_o/S_e = \frac{\mu_{max}}{FY}(X)(d)(a)(H)Z$$

predict essentially the same substrate removal when the mass of active organisms in each reactor is equal to that in one unit depth of plug-flow reactor.

(2) The observed and predicted removal were in good agreement. The correlation coefficient between the observed results and theoretical values using the plug-flow model was 0.96.

(3) Fixed film reactors respond rapidly to changes in substrate concentration and steady-state design equations would, therefore, introduce little error.

(4) The proposed plug-flow design model may be simplified to produce an equation suitable for field use in the design of trickling filters. This equation is

$$(S_o - S_e) + K_g \ln S_o/S_e = P/F\ (a)(H)Z$$

X. RECOMMENDATIONS

Although it has been demonstrated that a rational basis exists for the design of biological filters, it is also recognized that this investigation was only an initial step in that direction. Much remains to be done before these systems can be designed and operated with confidence. It is, therefore, recommended that the single stage studies be extended to determine

(1) The relative substrate and dissolved oxygen concentration at which dissolved oxygen becomes growth limiting.

(2) The effect of temperature in fixed film reactors.

(3) The effect of velocity on the valve of the saturation constant K_g over an extended range of values.

(4) The relative resistance provided by liquid film diffusion, biological film diffusion and the biological reaction in the overall process.

Based on this report and the additional single stage studies listed above it is also recommended that:

(1) Pilot studies be run on packed towers to check the validity of the proposed design equation.

(2) That the effect of recirculation be incorporated into the basic model. This is dependent upon defining the saturation constant as a function of velocity.

(3) That a procedure be developed for optimizing the design of biological filters based on the equation incorporating recycle and the effect of velocity or hydraulic loading on the saturation constant K_g.

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APPENDIX

APPENDIX I

GLOSSARY

GLOSSARY

An effort has been made during this investigation to maintain uniformity by using symbols recommended by Dr. Z. Fencl in Folia Microbiologica (1963). However, slight modifications or additions were required to satisfy the requirements of this investigation. Furthermore, the symbols listed below pertain only to the original portion of this manuscript and the symbols used in the literature review were those employed in the original work with minor exceptions.

Symbol	Description
A	Surface area of biological film (L^2)
a	Specific surface area of filter media (L^2/L^3)
C_o	Dye concentration obtained by dividing the injected mass by the reactor volume $V(M/L^3)$
C	Instantaneous dye concentration of an incremental volume of fluid (M/L^3)
d	Active thickness of biological film (L)
E	Removal efficiency (%)
F	Hydraulic flow rate (L^3/T)
f	Fraction of organic removal due to maintenance
H	Cross sectional area of a trickling filter (L^2)
h	Total thickness of biological film (L)
K	A constant (M/L^3)
K_g	Essential nutrient or substrate concentration where $\mu = 1/2\ \mu_{max}(M/L^3)$

Symbol	Description
k_m	Maintenance coefficient (T^{-1})
M_o	Mass of organisms in the active layer (M)
M_s	Mass of substrate or essential nutrient (M)
n	Number of reactors in series
P	Area capacity constant and equal to $\frac{1}{Y}$ (μ_{max})(d)(X) (M/L^2T)
R	Substrate removal rate for the total system (M/T)
R_1, R_2, R_n	Substrate removal rate for reactor 1 through n (M/T)
$S_1, S_2, \ldots, S_n$	Substrate concentration in reactor 1 through n (M/L^3)
S_o -	Influent substrate concentration (M/L^3)
S_e	Effluent substrate concentration from a plug-flow reactor (M/L^3)
T	Actual retention time of an incremental volume of liquid (T)
T_R	Theoretical liquid retention time (T)
t	Time (T)
μ	Specific growth rate (T^{-1})
μ_{max}	Maximum specific growth rate (T^{-1})
V	Reactor volume (L^3)
X	Unit mass of biological film on a dry weight basis (M/L^3)
Y	Apparent growth yield
Y_g	True growth yield
Z	Depth of trickling filter (L)

APPENDIX 2

DERIVATIONS

THE EFFECT OF SUBSTRATE CONCENTRATION AND MAINTENANCE ENERGY ON THE APPARENT YIELD, Y

Throughout this manuscript theoretical equations were derived in which no distinction was made between substrate removal due to growth and that required for maintenance of the existing cells. This does not mean that substrate removal due to cell maintenance was neglected. It simply implies that substrate removal due to cellular growth and maintenance were incorporated through the use of an apparent growth yield, Y.

Pirt (97) showed that the total substrate utilization represents the sum of that required for growth and maintenance or

$$(ds/dt)\ \text{utilization} = (ds/dt)\ \text{growth} + (ds/dt)\ \text{maintenance} \qquad (82)$$

This may be expressed in terms of organism growth through the equation

$$\frac{\mu}{Y}(X) = \frac{\mu}{Y_g}(X) + k_m X \qquad (83)$$

As Pirt (97) showed the apparent yield, true yield and maintenance coefficient may then be related through the expression

$$\frac{\mu}{Y} = \frac{\mu}{Y_g} + k_m \qquad (84)$$

or

$$\frac{1}{Y} = \frac{1}{Y_g} + \frac{k_m}{\mu} \qquad (85)$$

The apparent yield Y will, therefore, provide a valid relation between substrate removal and organism growth when (1) the maintenance coefficient has a negligible value, or (2) when substrate removal due to maintenance requirements utilizes a constant fraction of the total removal, regardless of the magnitude of this removal.

It may be shown that the fraction of substrate removal due to maintenance of the existing population is

$$f = \frac{k_m}{\left[\frac{\mu}{Y_g} + k_m\right]} \qquad (86)$$

or

$$f = \frac{k_m}{\frac{(\mu_{max} S)}{Y_g K_g + S} + k_m} \qquad (87)$$

A series of true yield coefficients calculated by Washington (98) for bacterial cultures varied from 0.19 to 0.35, mg of cells/mg of substrate. The maximum specific growths rates during these studies ranged from 0.7 hr^{-1} to 1.2 hr^{-1} and the maintenance coefficient ranged from 0.01 hr^{-1} to 0.067 hr^{-1}. Based on a true yield coefficient of 0.25 mg cells/mg substrate and a maintenance coefficient of 0.03 hrs^{-1} the percent substrate removal due to maintenance at various substrate concentrations is shown in Figure 41 for maximum growth rates of

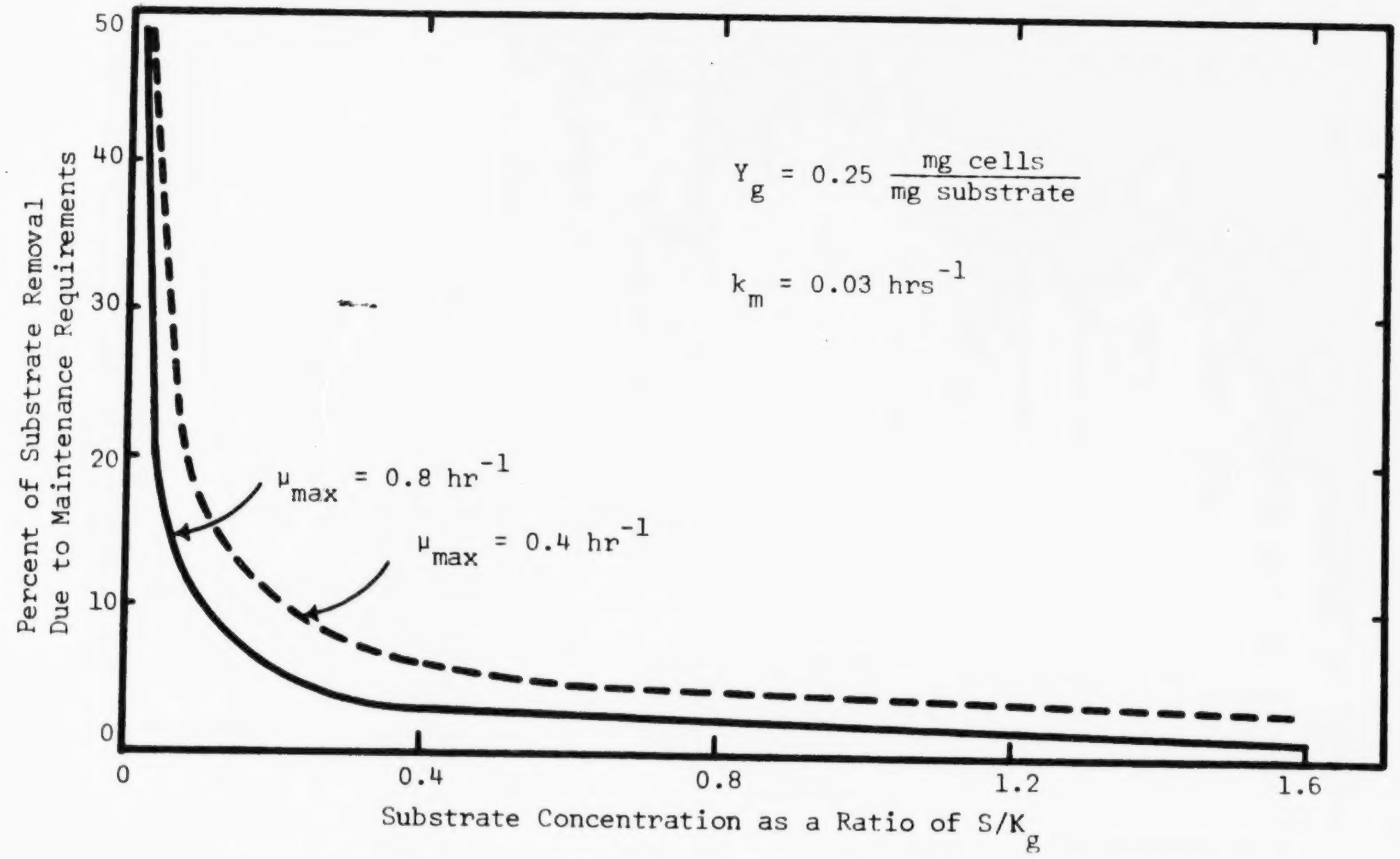

FIGURE 41. THE EFFECT OF SUBSTRATE CONCENTRATION ON THE PERCENTAGE OF THE REMOVED SUBSTRATE UTILIZED FOR MAINTENANCE

$0.4\ hr^{-1}$ and $0.8\ hr^{-1}$. The substrate is expressed as a function of the saturation constant K_g. From an examination of Figure 41 it is obvious that the change in the percent of substrate removal due to maintenance requirements is small until the substrate concentration drops below $0.2\ K_g$. Therefore, the average yield coefficient Y would vary only slightly and result in a negligible error. This point is demonstrated more clearly in Figure 42 where the apparent yield, Y is shown as a function of the substrate concentration. Here again, the substrate concentration is expressed as the ratio of the actual substrate concentration to the saturation constant K_g.

From Figure 42 it is seen that the apparent yield Y is essentially constant until the ratio S/K_g falls below 0.20. At a maximum specific growth rate of $0.8\ hr^{-1}$ the apparent yield increased from 0.237 mg cells/mg substrate to 0.245 mg cells/mg substrate as the ratio S/K_g went from 0.2 to 2.0. For a maximum specific growth rate of $0.4\ hr^{-1}$ the change in the apparent yield over the same substrate range was from 0.224 to 0.245. In this set of experiments the substrate concentration was never below $0.2\ K_g$. Therefore, the use of the apparent yield constitutes a valid parameter for describing the substrate removal.

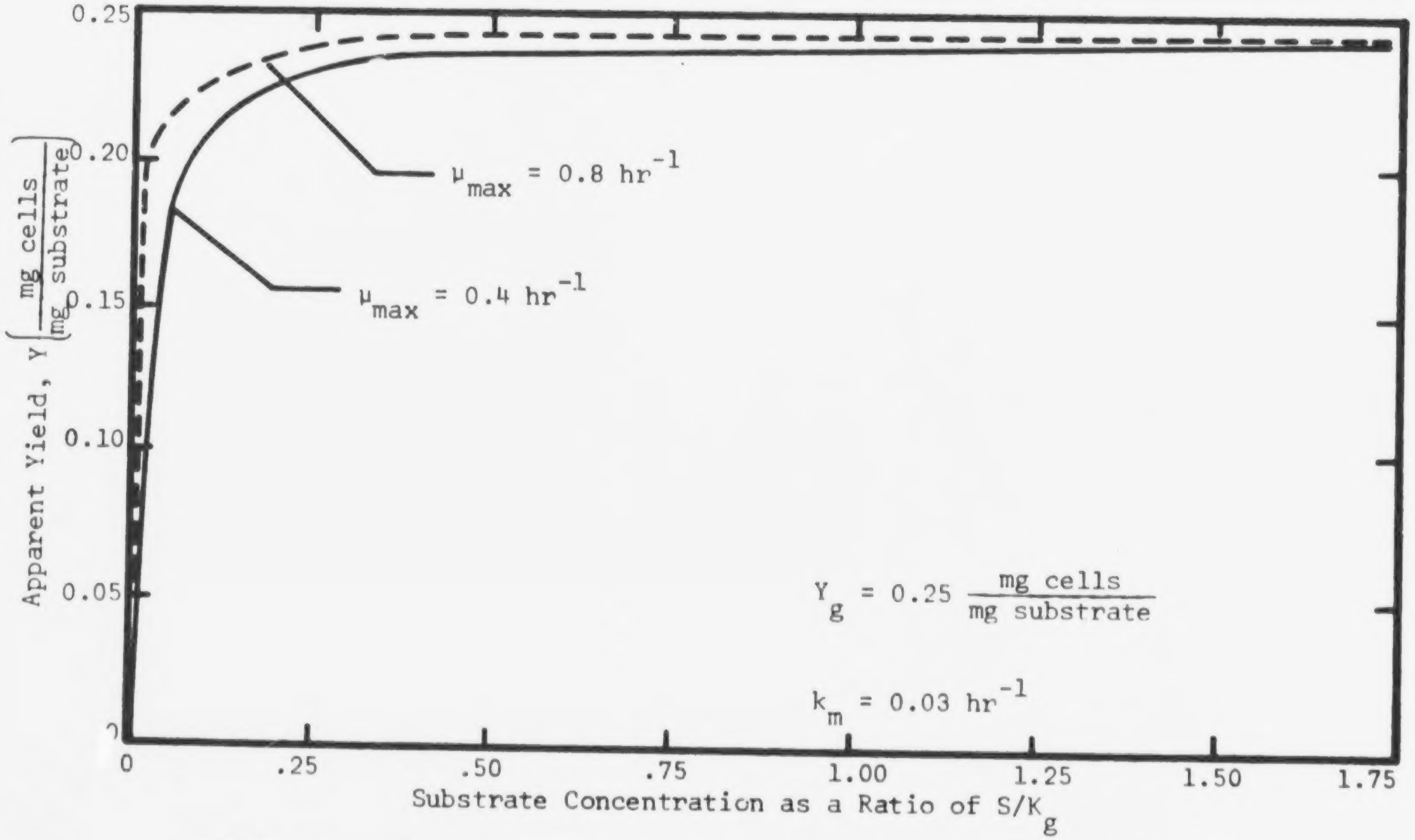

FIGURE 42. THE EFFECT OF SUBSTRATE CONCENTRATION ON THE APPARENT YIELD

APPENDIX 3

PROCEDURES

THE PREPARATION OF THE CONCENTRATED SUBSTRATE

The concentrated substrate was prepared by the procedure given below. The procedure must be carefully followed in the sequence given in order to avoid precipitation of salts. The following instructions are for the preparation of one liter of concentrated substrate.

1. Tare container
2. Add 33.30 ml of trace salt solution prepared below
3. Add 550 ml of water
4. Add each of the following with mixing. Do not add compound until previous compound is in solution

a)	KH_2PO_4.	13.61 gms.
b)	NaH_2PO_4,H_2.	8.28 gms.
c)	$(NH_4)2SO_4$	5.28 gms.
d)	NH_4Cl	49.2 gms.
e)	$CaCl_2$	4.44 gms.
f)	$MgCl_2,6H_2O$	8.13 gms.
g)	Glucose	144.0 gms.

5. Dissolve 3.60 gms. yeast extract in 100 ml of water, with heating and stirring. Do not allow to boil. When cool, add to substrate container.

6. Add water to solution to make 1000 grams.

   a. Trace Salt Solution

   The trace salts and the complexing agent, sodium citrate, are prepared in a solution 240 times their strength in the diluted substrate.

The trace salts are added to distilled water in amounts given below and made to two liters with distilled water. The solution must be stirred until all salts are in solution.

Compound	Mass, Grams
$FeCl_3$	38.88
$MnCl_2.4H_2O$	9.48
$ZnCl_2$	6.54
$CuCl_2.2H_2O$	4.10
$CoCl_2.6H_2O$	5.71
$(NH_4)6Mo_7O_{24}.4H_2O$	4.15
$Na_2B_4O_7.10H_2O$	2.29
Na_3Citrate	353.0

Note: When prepared as above, salt solution will have very dark color; however, with adequate stirring all salts will go into solution.

Compounds should be added in the order shown above, and each compound should not be added until previous compound is in solution.

TOTAL AND VOLATILE SUSPENDED SOLIDS DETERMINATIONS USING GLASS FIBER FILTERS

1. Grasp two glass fiber filters[a] with clean forceps and wash in distilled water for several seconds to remove lose fibers. Two filters are used for added rigidity and ease of handling.

2. Press the wet filters between two paper towels to remove excess moisture.

3. Lay the filters on cupped aluminum screens and place the screens in a 103°C oven for 3-4 hours to dry.

4. When dry, place the filters in glass desiccators[b] until needed. Each of the individual glass desiccators must be numbered.

5. Weigh[c] the filters and record the weight by desiccator number. The weight should be read exactly 35 seconds after the filters are removed from the desiccator.

6. Apply the filters to the center support of a fritted glass vacuum support[d] and secure with a few drops of distilled water.

7. Clamp the vacuum filter apparatus together, add the sample and apply the vacuum. When possible use an aliquot large enough to

---

[a] Reeve-Angle Glass Fiber Filters-55 mm diameter.

[b] Fisher Ointment Jar No. 11-873.

[c] Mettler Semi-Micro Gram-atic.

[d] Millipore Pyrex Holder No. xx 10-047-00.

produce a weight increase of at least 10 mg of solids.

8. After the sample is completely filtered, wash the funnel with distilled water and allow it to filter through the solids.

9. Carefully remove the filters from the fritted glass supports and place them on the cupped aluminum screens.

10. Place the filters and screen into a 103°C oven for approximately 4 hours to dry.

11. Remove the screens and filters to individual aluminum desiccators[e].

12. Weigh the filters and record the weight. The weight shall be read 35 seconds after removing the filters from the aluminum desiccators.

13. Transfer the filters only to numbered crucibles and fire at 520°C for 30 minutes.

14. Remove the filters from the crucibles and place them on the screens in the individual aluminum desiccators.

15. Allow the filters to cool for 30 minutes and then determine the weight 35 seconds after removing the filters from the desiccators.

---

[e] Fisher Desiccator No. 8-592.

SAMPLE PRESERVATION USING SATURATED MERCURIC CHLORIDE

During this experiment substrate samples were often collected so frequently that an analysis could not be made between tests. Therefore, a method of preserving test samples was required. Freezing was originally tried but was found to be unsatisfactory. Sample degradation was noted during the freezing and thawing cycle. Therefore, the addition of a small quantity of a saturated solution of mercuric chloride was tried. This was found to prevent microbial growth and sample degradation, but interfered with the test method.

In order to delineate the magnitude of the error the apparent reduction resulting from various mercuric chloride additions was studied. Six cell free samples containing 50 mg/1 of glucose were treated to produce samples having from zero to 3.6 percent by volume of the saturated mercuric chloride solution. As shown in Table 7 the reduction in the apparent glucose concentration was proportional to the volume of the mercuric chloride solution added. This is demonstrated more vividly in Figure 43 which shows the glucose concentration as a percent of the actual concentration versus the saturated mercuric chloride solution added as a percent by volume. A linear relation obviously exists.

In addition to defining the error produced by saturated HgCl solutions it was also necessary to define the addition required to prevent sample degradation. This was accomplished by adding various

TABLE 7

INTERFERENCE TEST FOR HgCl AS A GLUCOSE PRESERVATIVE

Drops of Saturated HgCl Added	% of Sample Volume	Glucose Concentration (Apparent)	% of Actual Value
0	0.000	50.0	100.00
1	0.235	48.8	97.6
2	0.468	47.8	95.6
4	0.932	45.3	90.6
8	1.848	42.5	85.0
16	3.623	34.8	69.6

17 Drops = 1.0 ml AgCl

Sample Volume = 25 ml

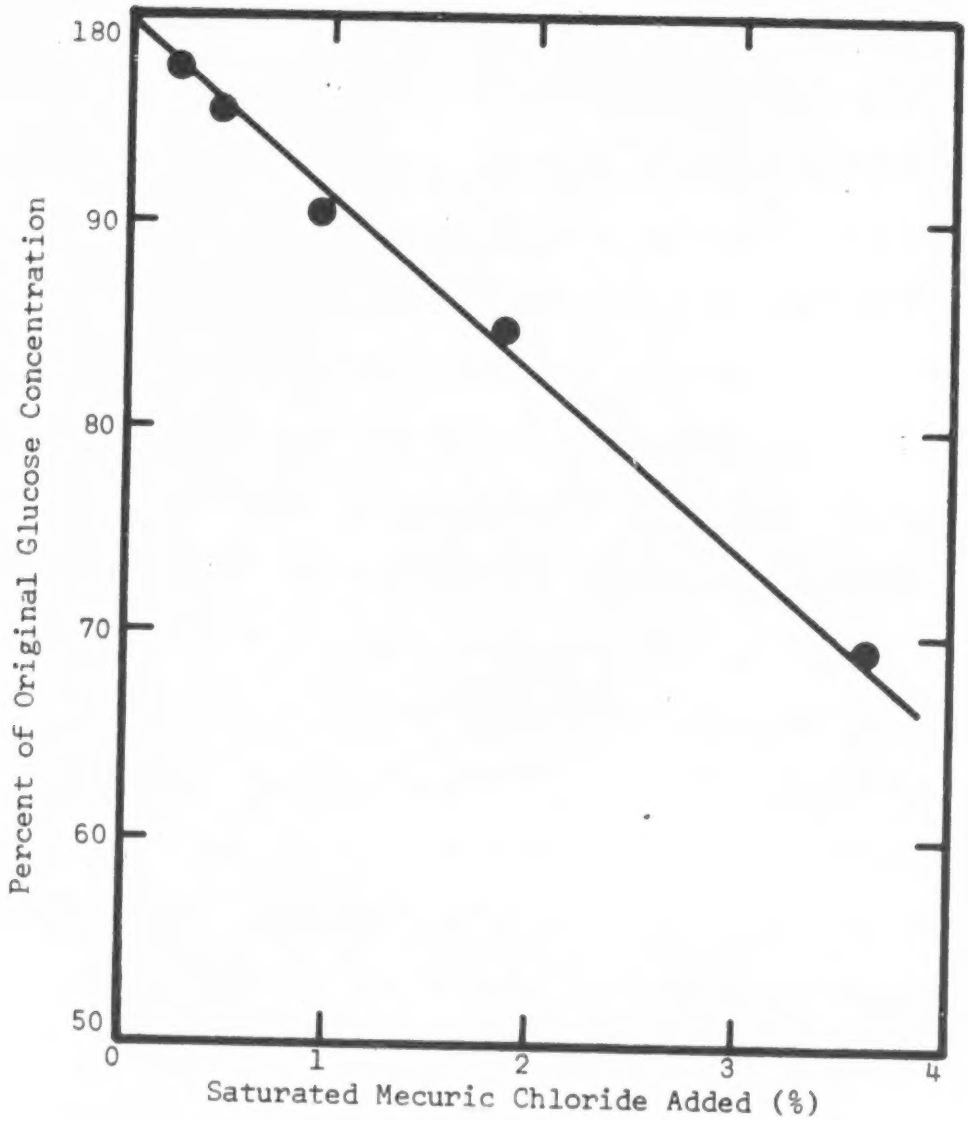

FIGURE 43. PERCENT OF ORIGINAL GLUCOSE CONCENTRATION VERSUS VOLUME PERCENT OF SATURATED HgCl SOLUTION ADDED

amounts of saturated mercuric chloride solution to samples containing an original glucose concentration of 92 mg/l and microbial cells. The glucose concentration was then determined 24 and 48 hours after sample preparation. The effectiveness of various mercuric chloride additions in preserving the sample is shown in Table 8. It should be noted that all samples had apparent glucose concentrations below the original concentration of 92 mg/l. In some cases this was due to interference by the mercuric chloride and in others a result of sample degradation. The distinction between interference and degradation may be demonstrated from Figure 44. Within reasonable limits the samples preserved with 0.12 percent saturated HgCl solution or higher showed the anticipated reduction in apparent glucose concentration. At concentrations below 0.12 percent mercuric chloride the glucose concentrations were much lower than predicted from the interference curve and indicated sample degradation. In the remainder of this experiment all samples were preserved with approximately 0.24 percent (2 drops/50ml) of saturated mercuric chloride solution and corrections were made for interference.

TABLE 8

PRESERVATION AND INTERFERENCE TEST FOR HgCl AS A PRESERVATIVE

Original Glucose Concentration = 92.2 mg/l
17.0 Drops = 1ml of HgCl
1 Drop was Added to each Sample

Sample Size	HgCl Concentration Volume %	Apparent Glucose Concentration	% of Actual Value	Remarks
		24-Hour Results		
5 ml	1.175	84.7 mg/l	92.0	Interference
25 ml	.235	88.2	95.7	Interference
50 ml	.117	90.8	98.5	Interference
125 ml	.047	87.6	95.0	Degradation
250 ml	.0235	82.4	89.4	Degradation
500 ml	.0117	71.5	77.6	Degradation
		48-Hour Results		
5 ml	1.175	84.9	92.1	Interference
25 ml	.235	88.1	95.7	Interference
50 ml	.117	90.8	98.5	Interference
125 ml	.047	87.7	95.1	Degradation
250 ml	.0235	82.2	89.3	Degradation
500 ml	.0117	65.9	71.5	Degradation

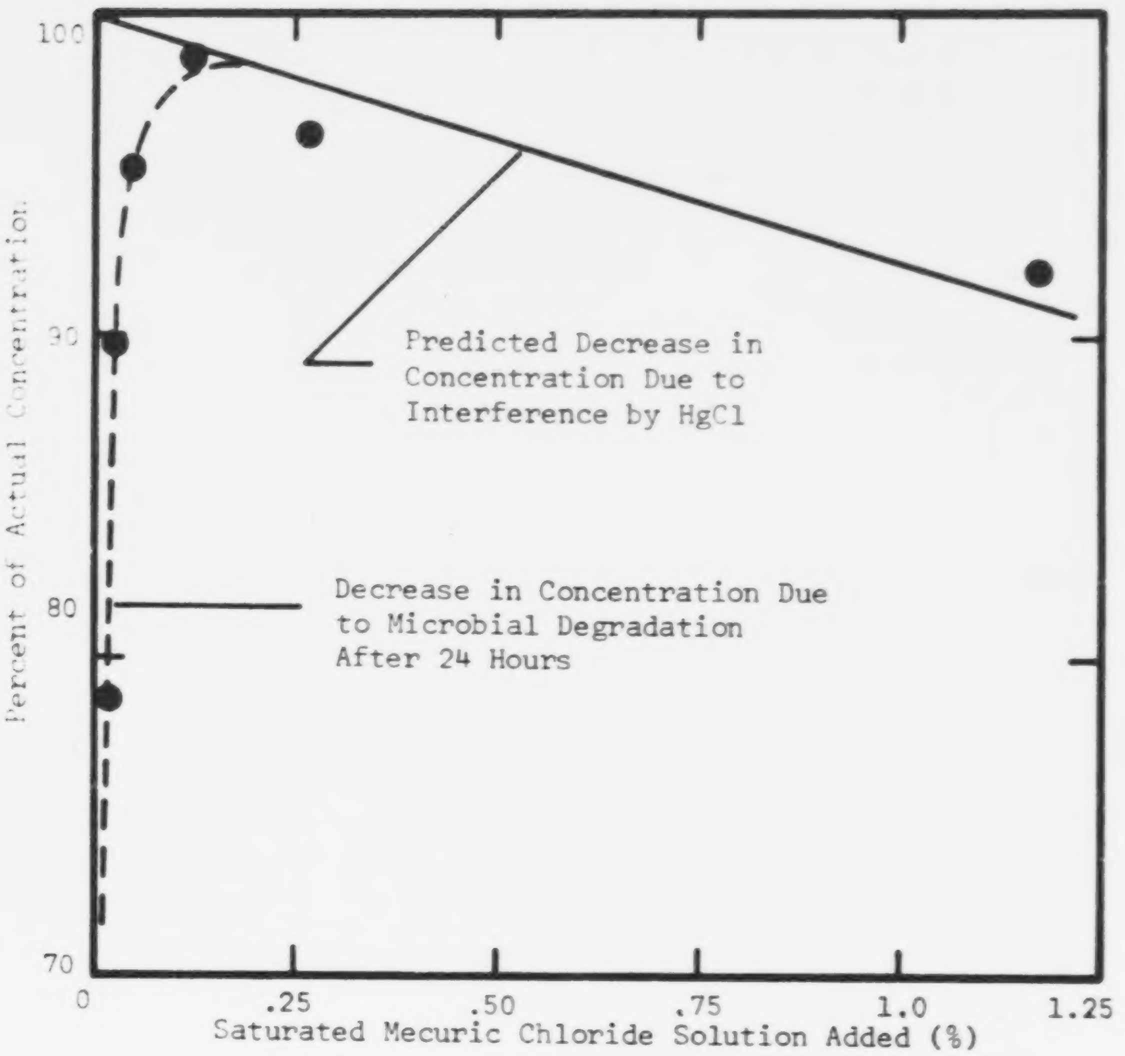

FIGURE 44. THE EFFECT OF SATURATED MECURIC CHLORIDE ADDITIONS ON THE PRESERVATION OF GLUCOSE SAMPLES

DISSOLVED OXYGEN PROBE RESPONSE AS A FUNCTION OF FLOW RATE AND CELL CONFIGURATION

Since the dissolved oxygen concentration was to be continuously monitored with a Beckman Model 778 D. O. Analyzer, considerable effort was directed toward the design of a D. O. cell. It was desirable to produce a cell configuration in which probe response was not velocity dependent at the design flow of 134 ml/min. To minimize resistance to diffusion through the liquid film the cell influent was positioned to impinge directly on the probe membrane. Both the cell configuration and probe response at various flow rates is shown in Figure 45.

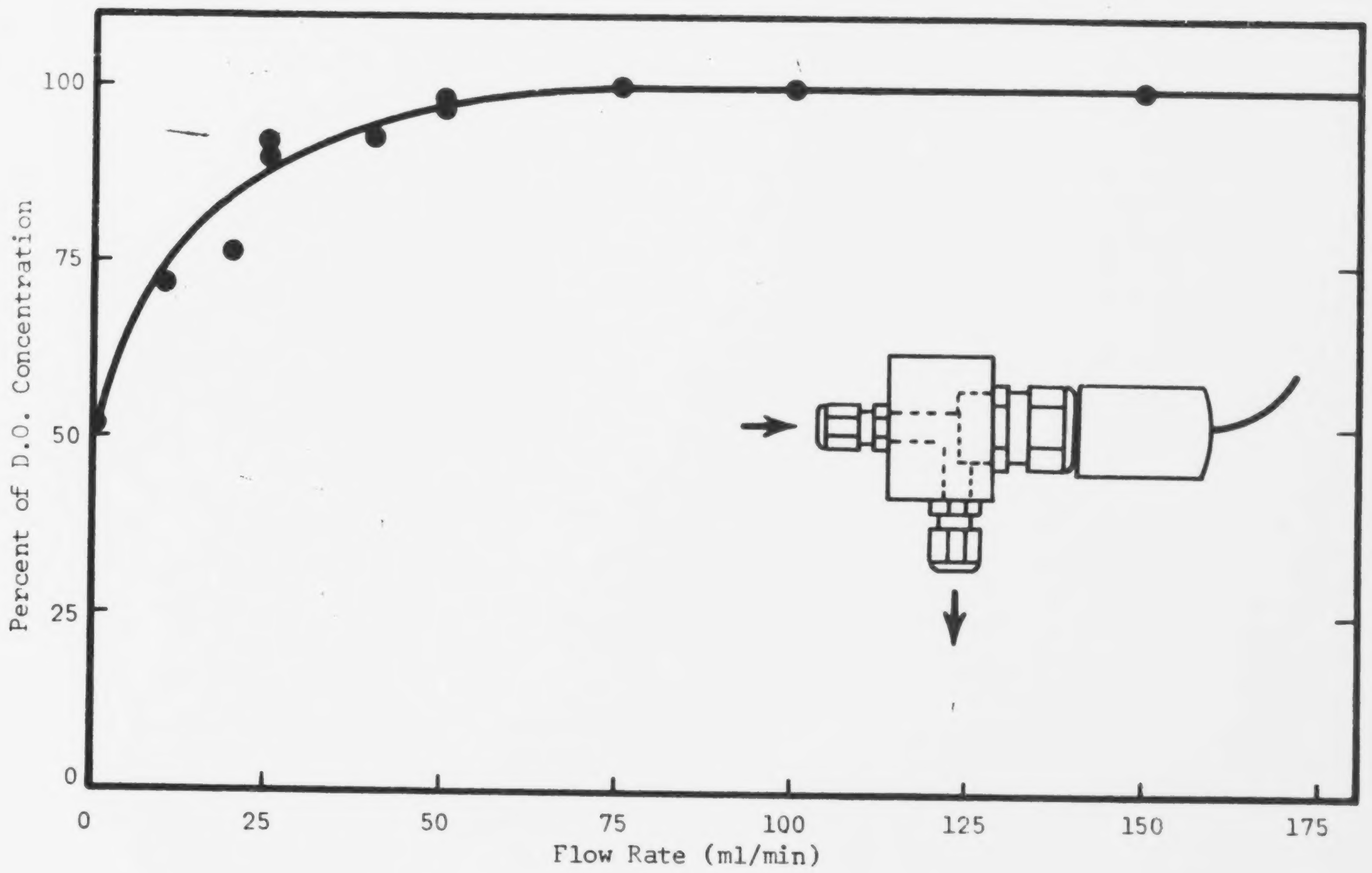

FIGURE 45. D.O. PROBE RESPONSE AS A FUNCTION OF FLOW RATE

APPENDIX 4

TABULAR RESULTS

TABLE 9

RESIDENCE TIME DISTRIBUTION DATA FOR A SINGLE REACTOR AT 25 RPM

V = 1000 ml

Residence Time = 7.4 min.

Flow = 134 ml/min

C_o = 0.046 mg/l

Time After Injection (min)	Dye Concentration (mg/l)	Reduced Time (T/TR)	Reduced Concentration (C/C_o)
0.70	0.086	0.09	2.061
1.40	0.038	0.18	0.845
1.90	0.040	0.25	0.885
4.00	0.026	0.54	0.582
6.00	0.018	0.81	0.402
8.00	0.012	1.10	0.280
10.00	0.009	1.35	0.202
12.00	0.006	1.41	0.180
16.00	0.003	2.16	0.067
20.00	0.002	2.70	0.043
24.00	0.000	3.24	0.019

Model III Turner Flourometer.
Rhodamine 6 GDN Extra Dye.
I-60-58 Primary Filter.
23A-Secondary Filter.
30X Sensitivity.
ΔV = 5 ml (1/200 V).
ΔT = 4.5 sec (1/100 Tr).

TABLE 10

RESIDENCE TIME DISTRIBUTION DATA FOR A SINGLE REACTOR AT 50 RPM

Volume = 1000 ml Residence Time = 7.41 min.

Flow = 135 ml/min C_o = 0.47 mg/l

Time After Injection (min)	Dye Concentration (mg/l)	Reduced Time T/T_r	Reduced Concentration C/C_o
0.30	0.086	0.04	1.830
0.60	0.038	0.08	0.806
1.00	0.045	0.13	0.958
2.00	0.035	0.27	0.756
4.00	0.026	0.54	0.553
6.00	0.018	0.81	0.395
8.00	0.014	1.11	0.308
10.00	0.011	1.35	0.234
12.00	0.008	1.62	0.183
16.00	0.005	2.16	0.117
20.00	0.003	2.70	0.074
24.00	0.002	3.24	0.042
30.00	0.001	4.05	0.031

Model III Turner Flourometer
Rhodamine 6 GDN Extra Dye
I-60-58 Primary Filter
23 A-Secondary Filter
30X Sensitivity
$\Delta V = 5\ \text{ml} \left(\frac{1}{200} V\right)$
$\Delta T = 4.5\ \text{sec} \left(\frac{1}{100} T_r\right)$

TABLE 11

RESIDENCE TIME DISTRIBUTION DATA FOR A SINGLE REACTOR AT 100 RPM

Volume = 1000 ml　　Residence Time = 7.4

Flow = 135 ml/min　　C_o = 0.046 mg/l

Time After Injection (min)	Dye Concentration (mg/l)	Reduced Time T/T_r	Reduced Concentration C_o
0.25	0.086	0.03	1.870
0.40	0.043	0.05	1.870
0.50	0.050	0.06	1.097
1.00	0.041	0.13	0.901
2.00	0.035	0.27	0.771
4.00	0.025	0.54	0.554
6.00	0.019	0.81	0.422
8.00	0.015	1.11	0.337
10.00	0.011	1.35	0.250
12.00	0.009	1.62	0.206
16.00	0.006	2.16	0.130
20.00	0.004	2.70	0.087
24.00	0.002	3.24	0.054

Model III Turner Flourometer
Rhodamine 6 GDN Extra Dye
I-60-58 Primary Filter
23 A-Secondary Filter
30X Sensitivity

$\Delta V = 5 \text{ ml} \left(\frac{1}{200} V\right)$

$\Delta T = 4.5 \text{ sec} \left(\frac{1}{100} T_r\right)$

TABLE 12

RESIDENCE TIME DISTRIBUTION DATA FOR A SINGLE REACTOR AT 200 RPM

Volume = 1000 ml Residence Time = 7.4

Flow = 135 ml/min C_o = 0.046 mg/l

Time After Injection (min)	Dye Concentration (mg/l)	Reduced Time T/T_r	Reduced Concentration C/C_o
0.10	0.070	0.01	1.525
0.20	0.047	0.02	1.032
1.00	0.039	0.13	0.847
2.00	0.033	0.27	0.725
4.00	0.024	0.54	0.533
6.00	0.018	0.81	0.402
8.00	0.015	1.11	0.326
10.00	0.011	1.35	0.239
12.00	0.007	1.62	0.157
16.00	0.004	2.16	0.098
20.00	0.002	2.70	0.054
24.00	0.001	3.24	0.032

Model III Turner Flourometer
Rhodamine 6 GDN Extra Dye
I-60-58 Primary Filter
23 A-Secondary Filter
30X Sensitivity

$\Delta V = 5 \text{ ml}\left(\frac{1}{200} V\right)$

$\Delta T = 4.5 \text{ sec}\left(\frac{1}{100} T_r\right)$

TABLE 13

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 50 RPM AND 6 MG/L D.O.

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/hr)	Film Thickness (μ)	Suspended Solids Total (mg/l)	Suspended Solids Volatile (mg/l)	Time* (hrs)
REACTOR 1							
208.0	200.0	8.0	63.2	10.0	1.2	1.2	0
200.0	174.0	26.0	205.5	29.0	-	-	8
201.0	128.0	73.0	576.8	91.0	30.0	27.8	21
210.0	132.0	78.0	616.4	314.0	-	-	32
196.5	128.5	68.0	537.3	484.0	25.7	22.8	48
REACTOR 2							
215.5	210.0	5.5	44.7	9.4	1.2	1.2	0
198.0	175.0	23.0	187.1	24.4	-	-	8
196.0	140.0	56.0	455.6	49.2	28.6	27.2	21
208.5	128.0	80.5	654.9	240.0	-	-	32
203.5	130.5	73.0	593.9	246.0	28.3	25.0	48
REACTOR 3							
196.0	204.0	-	-	10.8	1.1	1.1	0
186.0**	174.0	12.0	97.6	9.2	-	-	8
196.0	142.5	53.5	435.0	35.2	28.1	25.1	21
187.0**	126.5	60.5	595.9	134.4	-	-	32
195.0	122.5	72.5	589.4	202.0	27.0	23.4	48

*Time after initiating continuous flow.

**Influent concentration was below limits of test (215 ± 20 mg/l) and was rejected.

TABLE 14

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 100 RPM and 6 MG/L D.O.

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/hr)	Film Thickness (μ)	Suspended Solids Total (mg/l)	Suspended Solids Volatile (mg/l)	Time* (hrs)
REACTOR 1							
221.0	196.8	24.2	192.3	18.0	-	-	1
220.0	165.0	55.0	435.9	44.6	-	-	8
216.5	136.0	80.5	638.0	74.6	25.2	22.5	26
223.0	132.5	90.5	717.3	198.8	-	-	55
REACTOR 2							
252.0**	229.4	22.6	185.7	21.0	-	-	1
251.5**	196.5	55.0	449.8	47.2	-	-	8
244.0**	150.5	93.5	764.6	97.4	29.1	26.4	26
239.0**	117.6	121.4	992.8	242.0	-	-	55
REACTOR 3							
222.0	200.6	21.4	172.8	19.0	-	-	1
221.0	161.5	59.5	478.7	50.2	-	-	8
212.0	129.0	83.0	667.8	155.0	26.2	23.4	26

*Time after initiating continuous flow.

**Influent concentration exceeded test limits (215 ± 20) and was rejected.

TABLE 15

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 150 RPM AND 6 MG/L D.O.

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/hr)	Film Thickness (μ)	Suspended Solids		Time* (hrs)
					Total (mg/l)	Volatile (mg/l)	
REACTOR 1							
208.0	192.8	15.2	120.0	7.6	1.0	1.0	8
218.4	166.0	52.0	410.0	15.0	19.4	17.5	28
218.4	59.4	159.0	1259.6	-	-	-	37
217.2	113.4	103.8	820.2	155.0	53.7	48.3	55
217.2	100.0	117.2	926.1	180.0	-	-	63
-	-	-	-	-	54.0	49.0	76
REACTOR 2							
209.2	222.4	-	-	7.2	<1.0	<1.0	8
209.2	184.0	25.2	200.6	16.8	21.8	19.3	28
209.2	132.0	77.2	629.0	-	-	-	37
224.0	123.6	100.4	818.1	130.0	43.6	38.6	55
224.0	118.0	106.0	852.2	160.0	-	-	63
-	-	-	-	-	47.3	42.0	76
REACTOR 3							
195.6	193.6	2.0	16.0	8.8	<1.0	<1.0	8
193.6	154.0	39.6	-	13.2	22.6	19.2	28
193.6	99.0	94.6	758.3	-	-	-	37
230.0	273.0	-	-	105.0	41.8	35.8	55
204.0	99.0	105.0	841.7	167.0	-	-	63
-	-	-	-	-	60.6	53.4	76

*Time after initiating continuous flow.

TABLE 16

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 200 RPM AND 6 MG/L D.O

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/hr)	Film Thickness (μ)	Suspended Solids		Time* (hrs)
					Total (mg/l)	Volatile (mg/l)	
REACTOR 1							
239.2	187.0	52.2	456.0	26.4	24.6	23.4	0
261.2**	185.4	75.8	614.0	-	-	-	8
261.2**	170.4	90.8	735.0	48.0	32.6	28.7	25
253.2**	144.4	108.8	884.0	65.2	64.9	59.0	33
253.2**	125.4	127.8	1039.0	109.0	29.8	27.1	51
REACTOR 2							
218.8	160.4	58.4	470.0	22.4	32.8	31.0	0
218.8	162.0	56.8	458.0	-	-	-	8
Sloughed	-	-	-	-	39.0	36.7	25
REACTOR 3							
204.0	165.0	39.0	314.0	30.8	23.3	21.6	0
208.0	153.0	55.0	443.0	-	-	-	8
208.0	103.0	105.0	845.0	71.2	51.8	48.3	25
194.8	95.6	99.2	815.0	105.4	58.8	54.2	33
195.2	92.4	102.8	835.0	84.4	46.9	42.4	51

*Time after initiating continuous flow.

**Influent concentration exceeded limits of test (215 ± 20 mg/l) and was rejected.

TABLE 17

SUBSTRATE REMOVAL AT VARIOUS MIXING SPEEDS FOR CONSTANT INFLUENT CONDITIONS

Mixing Speed (rpm)	Reactor No. 1				Reactor No. 2			
	Influent (mg/l)	Effluent (mg/l)	Removal (mg/l)	Removal Rate (mg/hr)	Influent (mg/l)	Effluent (mg/l)	Removal (mg/l)	Removal Rate (mg/hr)
200	200.8	87.6	113.2	897.9	210.5	97.4	113.1	905.9
175	200.8	93.6	107.2	850.3	210.5	102.2	108.3	867.5
150	200.8	99.2	101.6	805.9	210.5	110.2	100.3	803.4
150	200.8	97.6	103.2	818.6	210.5	108.0	102.5	821.0
125	200.8	105.0	95.8	759.9	210.5	115.6	94.9	760.1
100	200.8	113.4	87.4	693.3	210.5	121.6	88.9	712.1
75	200.8	111.2	89.6	710.7	210.5	125.6	84.9	680.0
50	200.8	116.8	84.0	666.3	210.5	126.2	84.3	675.2
25	200.8	154.4	46.4	368.0	210.5	151.6	58.9	471.8
200	200.8	99.2	101.6	805.9	210.5	99.0	111.5	893.1
100	200.8	108.6	92.2	731.3	210.5	110.4	100.1	801.8
50	200.8	112.4	88.4	701.2	210.5	125.0	85.5	684.9

TABLE 18

DISSOLVED OXYGEN CONCENTRATIONS AT VARIOUS MIXING SPEEDS FOR CONSTANT INFLUENT CONDITIONS

	REACTOR NO. 1		REACTOR NO. 2	
	Effluent Concentration		Effluent Concentration	
Mixing Speed (rpm)	Replicate (mg/l)	Average (mg/l)	Replicate (mg/l)	Average (mg/l)
200	Trace	Trace	Trace	Trace
175	Trace	Trace	Trace	Trace
150	2.20 2.00	2.10	2.60 2.55	2.57
150	1.15 1.20	1.17	Trace	Trace
125	6.40 6.20	6.30		1.60
100	7.55 7.35	7.45	5.85 6.00	5.93
75	7.50 9.40	8.45	8.40 9.40	8.30
50	Trace Trace		Trace Trace	
25	11.30 11.40	11.35	12.40 12.40	12.40
200	No D.O. Samples Taken			
100	No D.O. Samples Taken			
50	13.60 13.40	13.50	11.70 12.10	11.90

The influent dissolved oxygen concentration was greater than 36 mg/l but an exact value could not be obtained due to sample degassing in the D.O. cell and manually collected samples.

TABLE 19

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 100 RPM AND 8 MG/L D.O.

Influent S_o (mg/1)	Effluent S_1 (mg/1)	Removal (S_o-S_1) (mg/1)	Removal Rate $F(S_o-S_1)$ (mg/1)	Film Thickness (μ)	Suspended Solids Total (mg/1)	Suspended Solids Volatile (mg/1)	Time* (hrs)
REACTOR I							
225.6	212.4	13.2	106.5	8.4	<0.1	<0.1	5
216.8	180.0	36.8	297.0	29.2	-	-	16
226.0	132.2	93.8	757.0	140.0	26.2	25.0	34
212.0	115.6	96.4	777.9	162.0	-	-	40
212.0	117.4	94.6	763.4	203.0	30.8	28.1	56
REACTOR 2							
236.8	228.0	8.8	71.0	7.0	<0.1	<0.1	5
232.4	207.0	25.4	205.0	15.6	-	-	16
226.8	135.0	91.8	740.8	162.0	17.0	14.8	34
268.0**	148.4	119.6	965.2	194.0	-	-	40
Sloughed							56
REACTOR 3							
223.2	216.8	6.4	52.0	6.4	<0.1	<0.1	5
230.0	196.5	33.5	272.4	31.0	-	-	16
221.2	133.6	87.6	712.2	154.0	29.1	26.8	34
289.6**	170.0	119.6	972.3	182.0	-	-	40
222.4	120.0	102.0	827.6	-	-	-	
218.4	124.4	94.0	764.2	196.0	35.1	30.7	56

*Time after initiating continuous flow.

**Influent concentration exceeded limits of test (215 ± 20 mg/1) and was rejected.

TABLE 20

FILM ACCUMULATION, SUBSTRATE REMOVAL, AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 100 RPM AND 15 MG/L D.O.

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/l)	Film Thickness (μ)	Total (mg/l)	Volatile (mg/l)	Time* (hrs)
REACTOR 1							
220.8	216.0	4.8	38.0	4.8	-	-	0
230.4	213.2	17.2	136.5	16.6	5.0	4.6	10
235.2	173.0	62.2	493.7	31.6	-	-	16
242.0**	153.4	88.6	703.3	59.0	-	-	23
210.0	117.8	92.2	731.9	181.6	39.2	36.1	37
204.8	108.8	96.0	768.4	197.2	-	-	47
221.2	140.4	80.8	641.4	197.0	39.2	35.9	59
REACTOR 2							
228.0	232.4	-	-	3.2	-	-	0
230.8	213.6	17.2	140.8	21.4	4.1	3.7	10
248.0**	178.0	70.0	572.9	34.2	-	-	16
235.2	146.0	89.2	730.0	66.6	-	-	23
246.0**	140.0	106.0	867.5	140.0	45.6	41.9	37
Sloughed	-	-	-	196.8	-	-	47
Sloughed	-	-	-	-	-	-	59
REACTOR 3							
217.2	220.0	-	-	2.0	-	-	0
234.8	213.6	21.2	170.8	22.0	5.1	4.6	10
235.2	171.0	64.2	517.3	42.2	-	-	16
232.0	140.2	91.8	739.7	94.2	-	-	23
230.0	131.6	98.4	792.9	185.0	38.9	36.2	37
218.0	124.0	94.0	757.4	215.8	-	-	47
232.0	122.2	109.8	884.8	204.0	43.0	39.0	59

*Time after initiating continuous flow.

**Influent concentration exceeded limits of test (215 ± 20 mg/l) and was rejected.

TABLE 21

FILM ACCUMULATION, SUBSTRATE REMOVAL AND VOLATILE SUSPENDED SOLIDS PRODUCTION AT 100 RPM AND 2.5 MG/L D.O.

Influent S_o (mg/l)	Effluent S_1 (mg/l)	Removal (S_o-S_1) (mg/l)	Removal Rate $F(S_o-S_1)$ (mg/hr)	Film Thickness (μ)	Suspended Solids Total (mg/l)	Suspended Solids Volatile (mg/l)	Time* (hrs)
REACTOR 1							
210.7	170.5	40.2	332.8	24.8	-	-	12
210.2	133.9	76.3	610.4	44.0	25.9	23.4	21
209.7	124.5	85.2	680.4	160.0	-	-	35
REACTOR 2							
198.7	147.0	51.7	410.0	30.0	-	-	12
200.0	107.4	92.8	740.2	62.5	27.4	24.1	21
200.4	108.7	91.5	732.4	183.2	-	-	35
REACTOR 3							
214.6	161.4	53.2	426.3	31.6	-	-	12
217.5	123.3	94.2	751.7	63.5	30.7	29.7	21
213.7	120.5	93.2	747.3	190.4	-	-	35

TABLE 22

SUBSTRATE REMOVAL AT VARIOUS DISSOLVED OXYGEN CONCENTRATIONS

	Glucose Concentrations and Utilization Rates					
Residual	Reactor 1			Reactor 3		
D. O. (mg/l)	INF (mg/l)	EFF (mg/l)	UTIL (mg/hr)	INF (mg/l)	EFF (mg/l)	UTIL (mg/hr)
18.4	220	125	764	219	144	605
11.2	217	129	710	216	124	739
7.1	214	115	797	214	125	716
5.8	212	112	804	215	126	716
2.5	210	114	770	216	129	700

NOTES:

a) Reactor No. 2 sloughed before sampling.

b) Film thickness.

	R_1	R_3
Initial (56 hrs*)	197 μ	204 μ
Final (65 hrs)	184 μ	208 μ

*Time after initiating continuous flow.

TABLE 23

SUBSTRATE REMOVAL AND SOLIDS PRODUCTION FOR VARIOUS SUBSTRATE CONCENTRATIONS

Sample	S_o (mg/l)	S_1 (mg/l)	S_o-S_1 (mg/l)	$F(S_o-S_1)$ (mg/hr)	T.S.S. (mg/l)	V.S.S. (mg/l)	Percent Volatile	Yield $\frac{VSS}{(S_o-S_1)}$
1-5	454.2	321.6	132.6	1058.1	34.8	32.0	92.1	.241
1-6	369.0	249.0	120.0	957.6	40.2	35.6	88.7	.296
1-7	104.0	57.7	46.3	369.5	12.9	12.1	94.2	.262
1-8	60.2	27.2	33.0	263.3	-	-	-	-
1-9	577.0	440.0	137.0	1093.3	-	-	-	-
1-10	Sloughed During Sample				-	-	-	-
2-5	408.6	286.0	122.6	991.9	35.9	32.9	91.6	.269
2-6	336.0	228.0	108.0	878.0	26.8	23.6	87.9	.219
2-7	106.0	51.0	55.0	443.1	11.5	11.2	96.2	.205
2-8	69.0	31.5	37.5	305.7	-	-	-	-
2-9	525.0	400.0	125.0	1016.3	-	-	-	-
2-10	228.0	119.2	108.8	884.5	26.6	25.0	94.33	.230
3-5	Flow Dropped Below Desired Level							
3-6	311.2	192.0	119.2	958.4	28.5	25.0	87.6	.210
3-7	93.4	44.9	48.5	389.9	16.9	15.5	92.0	.319
3-8	59.2	27.0	32.2	258.9	-	-	-	-
3-9	Reactor Partially Sloughed				-	-	-	-
3-10	190.0	100.4	89.6	720.4	30.2	28.8	94.7	.322
				Average	26.4	24.2	91.9	.257

Temperature = 25°C, pH = 6.5, D.O. = 6 - 9 mg/l
Film Thickness = 200 ± 10 μ.

TABLE 24

SUBSTRATE UTILIZATION DATA FOR THE DETERMINATION OF μ_{max} AND K_g FOR A SINGLE STAGE REACTOR

S_o (mg/l)	S_1 (mg/l)	$1/S_1$ (hr/mg)	$F(S_o-S_1)$ (mg/hr)	$\frac{1}{F(S_o-S_1)}$ (hr/mg)
454.2	321.6	.00311	1058.0	.000945
369.0	249.0	.00401	957.6	.001040
104.0	57.7	.01730	369.5	.002706
60.2	27.2	.03680	263.3	.003798
577.0	440.0	.00227	1093.3	.000915
408.0	286.0	.00350	991.9	.001008
336.0	228.0	.00439	878.0	.001139
106.0	51.0	.01961	443.1	.002257
69.0	31.5	.03175	305.7	.003268
525.0	400.0	.00250	1016.3	.000984
228.0	119.2	.00893	884.5	.001131
311.0	192.0	.00521	958.4	.001043
93.4	44.9	.02227	389.9	.002565
59.2	27.0	.03704	258.9	.003862
190.0	100.4	.00996	720.4	.001388

Mixing Speed = 100 rpm
D. O. = 6 - 9 mg/1

TABLE 25

RESIDENCE TIME DISTRIBUTION DATA FOR SIX REACTORS IN SERIES

Theoretical				Actual			
Time (min)	T/T_r	C(mg/l)	C/C_o	Time (min)	T/T_r	C(mg/l)	C/C_o
0.00	0.0	0.0000	0.000	0.00	0.0	0.0000	0.000
0.75	0.1	0.0004	0.002	0.75	0.1	0.0000	0.000
1.50	0.2	0.0062	0.004	1.50	0.2	0.0000	0.000
3.00	0.4	0.0602	0.361	3.00	0.4	0.0165	0.280
4.50	0.6	0.1380	0.828	4.50	0.6	0.0415	0.705
6.00	0.8	0.1750	1.050	6.00	0.8	0.0560	0.950
7.50	1.0	0.1600	0.960	7.50	1.0	0.0560	0.950
9.00	1.2	0.1200	0.720	9.00	1.2	0.0465	0.790
10.50	1.4	0.0782	0.468	10.50	1.4	0.0325	0.551
12.00	1.6	0.0454	0.275	12.00	1.6	0.0220	0.373
13.50	1.8	0.0249	0.149	13.50	1.8	0.0180	0.305
15.00	2.0	0.0127	0.0761	15.00	2.0	0.0075	0.127
C_o = .1667 mg/l V = 6L F = 800 mg/l T_r = 7.5 min				C_o = .059 mg/l V = 6L F = 800 mg/l T_r = 7.5 min			

TABLE 26

RESIDENCE TIME DISTRIBUTION DATA FOR PACKED TOWERS

Reduced Data for an 18' Deep Filter Containing Polygrid Media[a]		Reduced Data for a 5.9 Foot Deep Filter Con- Taining Glass Spheres[b]	
Reduced Time	Reduced Concentration	Reduced Time	Reduced Concentration
0.00	0.00	0.00	0.00
0.23	0.00	0.28	0.00
0.47	0.31	0.38	0.09
0.70	1.14	0.48	0.68
0.93	1.22	0.54	1.36
1.16	0.76	0.64	1.59
1.40	0.40	0.70	1.36
1.64	0.18	0.85	1.02
1.86	0.12	1.01	0.68
2.09	0.08	1.28	0.34
2.32	0.03	1.71	0.11
2.56	0.01	2.56	0.05
2.79	0.00	3.42	0.00

a. Calculated from data presented by Eckenfelder and Barnhart (101).

b. Calculated from data presented by Sinkoff et al. (34).

TABLE 27

THE TRANSIENT RESPONSE OF SIX ANNULAR REACTORS IN SERIES TO STEP CHANGES IN INFLUENT SUBSTRATE CONCENTRATION

Step Changes Made Were from 525 mg/l to 400 mg/l
and from 400 mg/l to 145 mg/l

Remarks	Time After Initiating Change (min)	Influent Substrate Concentration* (mg/l)	Effluent Substrate Concentration (mg/l)
Initial Steady State Condition	-	525	390
Step Change	0.0	525	390
	7.5	400	470
	15.0	400	285
	22.5	400	278
	30.0	400	256
Additional Sample	60.0	400	273
Step Change	0	400	273
	7.5	145	146
	15.0	145	68
	22.5	145	62
	30.0	145	66
Additional Sample	60.0	145	65

*Average of one or more influent values.

TABLE 28

THEORETICAL AND OBSERVED SUBSTRATE CONCENTRATIONS FOR AN AVERAGE INFLUENT CONCENTRATION OF 746 mg/l

	Observed			Theoretical	
Reactor Number	Run 12A (mg/l)	Run 12B (mg/l)	Average (mg/l)	Plug-Flow Model (mg/l)	Series Reactor Model (mg/l)
Inf	742.0	750.0	746.0	746.0	746.0
1	719.0	720.0	719.5	720.3	720.5
2	685.0	690.0	687.5	695.0	695.0
3	636.0	650.0	643.0	669.7	669.7
4	646.0	636.0	641.0	644.4	644.6
5	622.0	615.0	618.5	619.2	619.6
6	615.0	590.0	602.5	594.2	594.8

TABLE 29

THEORETICAL AND OBSERVED SUBSTRATE CONCENTRATIONS FOR AVERAGE INFLUENT CONCENTRATION OF 273 mg/l

Reactor Number or Unit Depth	Observed			Theoretical	
	Run 12C (mg/l)	Run 12D (mg/l)	Average (mg/l)	Plug-Flow Model (mg/l)	Series Reactor Model (mg/l)
Inf	280.8	265.2	273.0	273.0	273.0
1	260.8	244.0	252.4	252.5	252.8
2	240.0	224.8	232.4	232.5	233.0
3	224.0	208.0	216.0	213.3	213.9
4	201.6	192.0	196.8	194.4	195.4
5	180.8	168.0	174.4	176.5	177.5
6	160.0	156.0	158.0	159.3	160.3

TABLE 30

THEORETICAL AND OBSERVED SUBSTRATE CONCENTRATIONS FOR AN AVERAGE INFLUENT CONCENTRATION OF 100 mg/l

Reactor Number or Unit Depth	Observed			Theoretical	
	Run 12E (mg/l)	Run 12F (mg/l)	Average (mg/l)	Plug-Flow Model (mg/l)	Series Reactor Model (mg/l)
Inf	101.0	99.0	100.0	100.0	100.0
1	88.4	84.0	86.2	87.1	87.5
2	74.0	74.2	74.1	75.1	76.0
3	58.0	56.0	57.1	64.3	65.5
4	54.0	51.6	52.8	54.5	56.0
5	41.2	37.2	39.2	45.8	47.6
6	33.4	28.0	30.7	38.2	40.2

TABLE 31

THE THICKNESS AND WET WEIGHT OF FILMS DEVELOPED AT VARIOUS MIXING SPEEDS

Mixing Speed (rpm)	Film Weight (gms)	Film Thickness (μ)
50	1.18	91
	1.45	49
	0.26	35
	2.55	314
	1.48	240
	0.79	127
	3.13	484
	2.01	246
	1.89	202
100	0.12	21
	0.20	22
	0.72	140
	1.51	185
	1.76	140
	1.16	162
	1.45	154
	1.80	162
	1.30	194
	1.80	192
	1.22	203
	1.84	196
150	0.42	28
	0.40	36
	0.44	34
	0.53	61
	0.51	50
	0.53	56
	1.01	161
	0.85	119
	0.94	124
	1.38	176
	1.11	138
	1.59	186
200	0.15	26
	0.16	22
	0.25	31
	0.37	48
	0.47	71

TABLE 32

SUBSTRATE REMOVAL DATA FOR THE DETERMINATION OF P AND K_g

Influent Concentration S_o (mg/l)	Observed Substrate Removal S_o-S_e (mg/l)	Effluent Concentration S_e (mg/l)	Ratio S_o/S_e
	Six Reactors in Series (Z=6)		
746	143.5	602.5	1.235
525	135.0	390.0	1.345
400	127.0	273.0	1.412
273	115.0	158.0	1.728
145	90.0	65.0	2.230
100	68.8	31.2	3.204
	Four Reactors in Series (Z=4)		
746	105.0	641.0	1.165
273	76.2	196.8	1.388
100	47.2	52.8	1.900
	Single Reactors (Z=1)		
746	26.5	719.5	1.036
273	20.6	252.4	1.080
100	13.3	86.2	1.155

ABSTRACT

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In spite of the advances made in the trickling filter process since its inception in 1889 the search for a more rational design procedure still continues. Progress in achieving such a design technique has apparently been hampered by an inadequate knowledge of the biological process. Since efficiency of design is contingent upon process knowledge, more basic studies are required.

In order to gain some insight into processes which depend upon biological films, a theoretical investigation was made of the kinetics of fixed-film, biological reactors. After several preliminary studies it was found that completely mixed, annular reactors would permit the greatest degree of control and measurements could be made with ease and accuracy. The annular reactors were innoculated with a mixed population of microorganisms and allowed to develop under batch conditions until attachment was obtained. The flow rate was then increased in increments over a 24-hour period until a retention time of 7.5 minutes was attained. At this low retention time the suspended organisms were washed out and any substrate (glucose) removal was then due to the film of microorganisms attached to the reactor walls.

Three reactors were operated in parallel to gain basic kinetic data and then six reactors in series were used to approximate the plug-flow conditions associated with trickling filters. During the basic kinetic studies the effect of mixing speed, dissolved oxygen and substrate concentration were studied. In the range of interest, it

was found that the rate of substrate removal was independent of the dissolved oxygen concentration, but was dependent on the liquid velocity and substrate concentration.

Although liquid film diffusion was important, it appeared that a diffusion model was an over-simplification of the process. Therefore, a saturation function which included the effects of both reaction and diffusion was incorporated with the continuous culture theory to derive mathematical models for single stage, multistage and plug-flow, fixed-film biological reactors. The validity of the theoretical models was supported with experimental evidence. Based on these results a trickling filter model was proposed and the application and significance of the model discussed.